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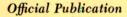
Inverican Journal OF OBSTETRICS AND GYNECOLOGY

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 Wynn V, Niththyananthan R: The effect of progestins in combined oral contraceptives on serum lipids with special reference to high-density lipoproteins. Am J Obstet Gynecol 142:766-772, 1982.
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ORAL CONTRACEPTIVE (O.C.) AGENTS

ORAL CONTRACEPTIVE (O.C.) AGENTS
Indications Prevention of pregnancy. DOSE-RELATED RISK OF THROMBDEM-BOLISM. Because studies have shown a positive association between OC estrogen dose and risk of thromboembolism, it is prudent to minimize estrogen exposure. Prescribe an OC with the least amount of estrogen compatible with an acceptable pregnancy rate and patient acceptance. Start new users on OCs containing 0.05 mg or less of estrogen.

Contraindications 1. Known or suspected pregnancy (see Warning #5). 2. Thrombophlebitis or thromboembolic disorders. 3. Past history of deep vein thrombophlebitis or thromboembolic disorders. 4. Undiagnosed abnormal gental bleeding. 5. OCs should not be used by women who have or have had any of the following: a. cerebral vascular or coronary artery disease; including myocardial infarction. b. known or suspected estrogen dependent neoplasia. d. benign or malignant liver tumor that developed during use of OCs or other estrogen containing products.

WARNINGS: Cigarette smoking increases the risk of serious cardiovascu-lar side effects from OC use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use OCs should be strongly advised not to smoke.

to smoke.

The use of OCs is associated with increased risk of several serious conditions including thromboembolism, stroke, myocardial infarction, liver tumor, gall bladder disease, visual disturbances, fetal abnormalities, and hypertension. Practitioners prescribing OCs should be familiar with the following information relating to these risks.

International prescripting out should be resumant with the behalf she there in the relating to these risks.

1. Thromboembolic Disorders and Other Vascular Problems: An increased risk of thromboembolic and thrombotic disease associated with OC use is established noe study demonstrated an increased relative risk for Itaal venous thromboembolism and several studies demonstrated it for non-Itaal venous thromboembolism. They estimate that OC users are 4-11 times more likely than nonusers to develop these diseases without evident cause. One British study reported an excess death rate of 40% in OC users, most of which resulted from cardiovascular diseases. Another British study showed a lower death rate in OC users that controls; an increase in cardiovascular disease was seen but was not statistically significant. A U.S. prospective study failed to disolose increased mortality rates from cardiovascular disorders in study showed significant increases in venous thromboembolism. CERE-BROVASCULAR DISORDERS. Two American studies demonstrated an increased relative risk for stroke not shown in prior British studies. In an American study and relative risk for stroke not shown in prior British studies in an American study and relative risk of hemorrhagic stroke was estimated as 2.0 times greater and thrombotic stroke as 4-95 times greater in users than nonusers. A British long-term follow-up study reported in 1976 a highly significant association between thrombotic stroke as 4-95 times greater in users than nonusers. A British long-term follow-up study reported in 1976 a highly significant association between the stroke as 4-95 times greater in users than nonusers. A British long-term follow-up study reported in 1976 a highly significant association between the survey of the properties of the second solution in 1974, but the number of cases was too small to estimate the risk. Subarachnoid hemorrhage is not the second of these accidents; smoking and pill use appear to increase silva more than either alone.

MYOCARDIAL INFARCTI

The process was too small to estimate the risk. Subarachnoid hemorrhage has been shown to be increased by CC use in British and American studies. Smoking alone increases incidence of these acidents; smoking and pill use appear to increase risk more than either alone.

MYOCARDIAL INRAPCTION (MI). Increased relative risk of MI associated with CC use has been reported. One British study found that the greater the number of underlying risk factors for coronary artery diseases (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of preedamptic toxemia) the higher the risk of developing MI. regardless of OC use. OCs were an additional risk factor. In terms of relative risk, it has been estimated that nonsmoking OC users (smoking) is considered a major predisposing condition to MI) are twice as likely to have a fatal MI as nonsmoking nonusers. OC users who are smokers have a 5-fold increased risk compared to nonsmoking users, and a 10-12-fold increased risk compared to nonsmoking users, and a 10-12-fold increased risk compared to morsmoking nonsmoking users, and a 10-12-fold increased risk compared to morsmoking nonsmoking users, and a 10-12-fold increased risk compared to nonsmoking users, and a 10-12-fold increased risk compared to nonsmoking users, and a 10-12-fold increased risk compared to morsmoking nonsmoking users, and a 10-12-fold increased risk compared to morsmoking nonsmoking users, and a 10-12-fold increased risk of various age groups must be considered (Estimates are based on believe the studies of the studies of the second studies and second second second second second secon

Age 40 + 30-39 Heavy smokers Light smokers AB D C,D Nonsmokers (other predisposing conditions) C.B

A-Use associated with very high risk.

B-Use associated with high risk.

C-Use associated with moderate risk.

D-Use associated with low risk.

Physician and patient should be alert to earliest manifestations of thromboembolic and thrombotic disorders (e.g. thrombophlebits, pulmonary embolism, centrovascular instifficency compary occlusion, retail thrombosis, and mesenteric thrombosis). Should arry of these occur or be suspected, discontinue Octorions has been reported in Octorions. If resistate previous previous properties are control to the property of the property occurs of the property occurs of the property occurs of the property occurs of post-supery thromboembolic complicators with contraceptive needs. Data of the property occurs occur

clinical significance is unknown. 8. Elevated Blood Pressure: An increase in blood pressure has been reported with Oct user hypertension may occur within a few months of beginning OCs. In the first year of user, incidence on hypertension may be no higher in OC users than in nonusers. Incidence in users increases with evaposure and in the first year of use is 25-3 times. In the first year of year of the control of the contr



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American Journal of Obstetrics and Gynecology

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PESCRIPTION

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routes. It is used for the management of pan not responsive to
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attendant loss of motor, sensory or sympathetic function.

attendant loss of motor, sensory or sympathetic function.

CONTRAINDICATIONS

DURAMORPH® PF is contraindicated in those medical conditions which would preclude the administration of opioids by the intravenous route—allergy to morphine or other opiates, acute bronchial asthma, upper airway obstruction.

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WARNINGS

WARNINGS

WARNINGS
DURAMORPH® PF administration should be limited to use by those familiar with the management of respiratory depression, and in the case of epidural or intrathecal administration, familiar with the techniques and patient menagement problems associated with epidural or intrathecal drug administration. Because epidural administration has been associated with lessened potential for immediate or late adverse effects than intrathecal administration, the epidural route should be used whenever possible. Rapid intravenous administration may result in chest wall rigidity.

FACILITIES WHERE DURAMORPH® PF IS ADMINISTERED MUST BE EQUIPPED WITH RESUSCITATIVE EQUIPMENT OXYGEN, NALOXONE INJECTION, AND OTHER RESUSCITATIVE DRUGS. WHEN THE EPIDURAL OR INTRATHECAL ROUTE OF ADMINISTRATION IS EMPLOYED, PATIENTS MUST BE OBSERVED IN A FULLY EQUIPPED AND STAFFED ENVIRONMENT FOR AT LEAST 24 HOURS.

SEVERE RESPIRATORY DEPRESSION UP TO 24 HOURS FOLLOW-ING EPIDURAL OR INTRATHECAL ADMINISTRATION HAS BEEN REPORTED.

Morphine sulfate may be habit forming. (See Drug Abuse and Dependence section.)

PRECAUTIONS GENERAL

GENERAL
Preservative-free DURAMORPH® PF (Morphine Sulfate Injection, USP) should be administered with extreme caution in aged or debilitated patients, in the presence of increased intra-cranial/intraocular pressure and in patients with head injury. Pupiliary changes (miosis) may, obscure the course of intracranial pathology Care is urged in patients who have a decreased respiratory reserve (e.g., emphysema, severe obesity, kyphoscoliosis).

Seizures may result from high doses. Patients with known seizure disorders should be carefully observed for evidence of morphineinduced seizure activity.

It is recommended that administration of DURAMORPH® PF by the epidural or intrathecal routes be limited to the lumbararea. Intrathecal use has been associated with a higher incidence of respiratory depression than epidural use.

tory depression than epidural use.

Smooth muscle hypertonicity may result in biliary colic, difficulty in urination and possible urinary retention requiring catheterization. Consideration should be given to risks inherent in urethral catheterization, e.g., sepsis, when epidural or intrathecal administration is considered, especially in the perioperative period.

Elimination half-life may be prolonged in patients with reduced metabolic rates and with hepatic or renal dysfunction. Hence, care should be exercised in administering morphine in these conditions, particularly with repeated dosing.

particularly with repeated dosing.

Patients with reduced circulating blood volume, impaired myoca dial function or on sympatholytic drugs should be observed care fully for orthostatic hypotension, particularly in transport. Patients with chronic obstructive pulmonary disease and patient with acute asthmatic attack may develop acute respiratory failur with administration of morphine. Use in these patients should b reserved for those whose conditions reguire endotracheal intubation and respiratory support or control of ventilation.

DRUG INTERACTIONS

DRUG INTERACTIONS

Depressant effects of morphine are potentiated by either concom
tant administration or in the presence of other CNS depressant
such as alcohol, sedatives, antihistaminics or psychotropic drug
(e.g., MAO inhibitors, phenothiazines, butyrophenones and tr
cyclic antidepressants). Premedication or intra-anesthetic use of
neuroleptics with morphine may increase the risk of respirator
depression. depression

CARCINOGENESIS, MUTAGENESIS, IMPAIRMENT OF FERTILIT'
Studies of morphine sulfate in animals to evaluate the carcinogeniand mutagenic potential or the effect on fertility have not beer conducted

PREGNANCY
Teratogenic effects—Pregnancy Category C. Animal reproduction studies have not been conducted with morphine sulfate. It is also not known whether morphine sulfate can cause fetal harm when administered to a pregnant woman or can affect reproduction capacity. Morphine sulfate should be given to a pregnant woman only if clearly needed.

Nonteratogenic effects. Infants born from mothers who have been

LABOR AND DELIVERY

LABOR AND DELIVERY
Intravenous morphine readily passes into the fetal circulation and may result in respiratory depression in the neonate. Naloxone and resuscitative equipment should be available for reversal of narcotic induced respiratory depression in the neonate. In addition, intra venous morphine may reduce the strength, duration and frequency of uterine contraction resulting in prolonged labor.

Epidurally and intrathecally administered morphine readily passes into the fetal circulation and may result in respiratory depression o

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the neonate. Controlled clinical studies have shown that *epidural* administration has little or no effect on the relief of labor pain.

administration has fitted or no effect of the relief of lader pain. However, studies have suggested that in most cases 0.2 to 1 mg of morphine intrathecally provides adequate pain relief with little effect on the duration of first stage labor. The second stage labor, though, may be prolonged if the parturient is not encouraged to bear down. A continuous intravenous infusion of naloxone, 0.6 mg/hr, for 24 hours after intrathecal injection may be employed to reduce the incidence of potential side effects.

NURSING MOTHERS

Morphine is excreted in maternal milk. Effect on the nursing infant is not known.

Safety and effectiveness in children have not been established.

ADVERSE REACTIONS

ADVERSE REACTIONS

The most serious side effect is respiratory depression. Because of delay in maximum CNS effect with intravenously administered drug (30 min), rapid administration may result in overdosing. Bolus administration by the epidural or intrathecal route may result in early respiratory depression due to direct venous redistribution of morphine to the respiratory centers in the brain. Late (up to 24 dours) onset of acute respiratory depression has been reported with administration by the epidural or intrathecal route and is believed to be the result of rostral spread. Reports of respiratory depression following intrathecal administration have been more frequent, but the dosage used in most of these cases has been considerably higher than that recommended. This depression may be severe and could require intervention (See Warnings and Overdosage sections). Even without clinical evidence of ventilatory inadequacy, a diminished CO₂ ventilation response may be noted for up to 22 hours following epidural or intrathecal administration.

While low doses of intravenously administered morphine have little

While low doses of intravenously administered morphine have little effect on cardiovascular stability, high doses are excitatory, resulting from sympathetic hyperactivity and increase in circulating catecholamines. Excitation of the central nervous system resulting in convulsions may accompany high doses of morphine given intravenously. Dysphoric reactions may occur and toxic psychoses have been reported.

Epidural or intrathecal administration is accompanied by a high incidence of pruritus which is dose related but not confined to site of administration. Nausea and vomiting are frequently seen in patients following morphine administration. Urinary retention which may persist for 10-20 hours following single epidural or intrathecal administration has been reported in approximately 90% of males. Incidence is somewhat lower in females. Patients may require catheterization (see Precautions). Pruritus, nausea/vomiting and urinary retention frequently can be alleviated by the intravenous administration of low doses of naloxone (0.2 mg).

Tolerance and dependence to chronically administered morphine, by whatever route, is known to occur (see Drug Abuse and Dependence section).

Miscellaneous side effects include constipation, headache, anxiety, depression of cough reflex, interference with thermal regulation and oliguria. Evidence of histamine release such as uticaria, wheals and/or local tissue irritation may occur.

In general, side effects are amenable to reversal by narcotic antag-onists. NALOXONE INJECTION AND RESUSCITATIVE EQUIPMENT SHOULD BE IMMEDIATELY AVAILABLE FOR ADMINISTRATION IN CASE OF LIFE-THREATENING OR INTOLERABLE SIDE EFFECTS.

DRUG ABUSE AND DEPENDENCE

Controlled Substance: Morphine sulfate is a Schedule II substance under the Drug Enforcement Administration classification.

Abuse: Morphine has recognized abuse potential

Dependence: Cerebral and spinal receptors may develop tolerance/dependence independently, as a function of local dosage. Care must be taken to avert withdrawal in those patients who have Care must be taken to avert windrawal in insige patients with have been maintained on parenteral/oral narrotics when epidural or intrathecal administration is considered. Withdrawal may occur following chronic epidural or intrathecal administration, as well as the development of tolerance to morphine by these routes. (See Nonteratogenic effects under Pregnancy.)

Overdosage is characterized by respiratory depression with or without concomitant CNS depression. Since respiratory arrest may result either through direct depression of the respiratory center or

as the result of hypoxia, primary attention should be given to the establishment of adequate respiratory exchange through provision of a patent airway and institution of assisted or controlled ventilation. The narcotic antagonist, naloxone, is a specific antidote. Naloxone (usually 0.4 mg) should be administered intravenously, simultaneously with respiratory resuscitation. As the duration of effect of naloxone is considerably shorter than that of epidural or intrathecal morphine, repeated administration may be necessary. Patients should be closely observed for evidence of renarcotization. Note: Respiratory depression may be delayed in onset up to 24 hours following epidural or intrathecal administration. In painful conditions, reversal of narcotic effect may result in acute onset of naloxone may permit reversal of side effects without affecting analgesia. Parenteral administration of narcotics in patients receiving epidural or intrathecal morphine may result in overdosage. ing epidural or intrathecal morphine may result in overdosage

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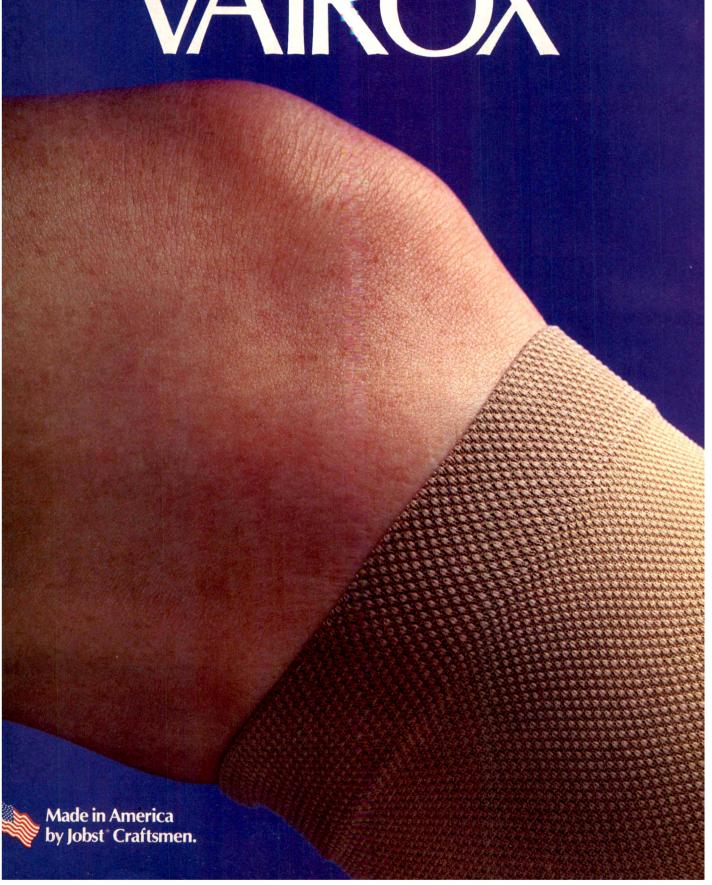
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 Cohen SE, Woods WA. Anesth 58:500, 1983
 Rawal N, Sjöstrand U, Christoffersson E, et al. Anesth Analg 63:583, 1984



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For further information please write:

Paul J. Meis, M.D.

Head. Section on Maternal-Fetal Medicine

01

Frank C. Greiss, Jr., M.D. Professor and Chairman

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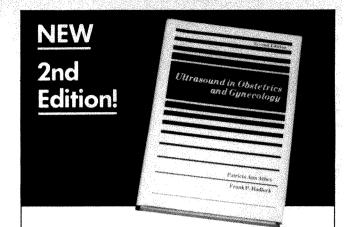
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References:

1. Rössner S et al: Acta Obstet Gynecol Scand 59:255, 1980.

2. Nash AL et al: Med J Atest 2:277, 1979, 3. Larsson-Cohn U et al: Fertil Steril 35:172, 1981, 4. Briggs MH: J Reprod Mea 28:92, 1983, 5. Ahrén T et al: Contraception 24:451, 1981.

6. Briggs MH, Briggs M: Acta Obstet Gynecol Scand Suppl 105:25, 1982.

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Contraindications—OC's should not be used in women with any of the Contraindications—OC's should not be used in women with any of the following conditions: 1. Thrombophiebits or thromboembolic disorders. 2. A past history of deep-vein thrombophiebitis or thromboembolic disor ders. 3. Cerebral-vascular or coronary-artery disease. 4. Known or suspected carcinoma of the breast. 5. Known or suspected estrogen-dependent neoplast. 6. Undiagnosed abnormal genital bleeding. 7. Known or suspected pregnancy (see Warning No. 5). 8. Benign or malignant liver tumor which developed during use of OC's or other estrogen-containing products. products Warnings

Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke. The use of oral contraceptives is associated with increaged risk of several serious conditions, including thromboembollsm, stroke, myocardial infarction, hepatic adenoma, galibladder disease, hypertension. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks.

1. Thromboembolic Disorders and Other Vascular Problems—An increased risk of thromboembolic and thrombotic disease associated with use of OC's is well established. Three principal studies in Great Britain and 3 in the U.S. have demonstrated increased risk of fatal and nonfatal venous thromboembolism and stroke, both hemorrhagic and thrombotic. These studies estimate that users of OC's are 4 to 11 times more likely than nonusers to develop these diseases without evident cause. CEREBROVASCULAR DISORDERS—In a collaborative American study of cerebrovascular disorders in women with and without predisposing causes, it was estimated that the risk of hemorrhagic stroke was 2.0 times greater in users than nonusers and the risk of thrombotic stroke was 4 to 9.5 times greater in users than in nonusers. MYOCARDIAL INFARCTION (MI)—An increased risk of MI associated with the use of OC's has been reported, confirming a previously suspected association. These studies, conducted in the UK, found, as expected, that the greater the number of underlying risk factors for coronary-artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of pre-eclamptic toxemia) the higher the risk of developing MI, regardless of whether the patient was an OC user or not. OC's, however, were found to be a clear additional risk factor. In 1. Thromboembolic Disorders and Other Vascular Problems - An risk of developing Mr., regardless of whether the patient was an OC user or not. OC's, however, were found to be a clear additional risk factor. In terms of relative risk, it has been estimated that OC users who do not smoke (smoking is considered a major predisposing condition to MI) are about twice as likely to have a fatal MI as nonusers who do not smoke. OC users who are also smokers have about a 5-fold increased risk of fatal MI compared to users who do not smoke, but about a 10- to 12-fold increased its compared to a possess who do not smoke.

OC users who are also smokers have about a 5-fold increased risk of fatal MI compared to users who do not smoke, but about a 10- to 12-fold increased risk compared to nonusers who do not smoke. Furthermore, amount of smoking is also an important factor. In determining importance of these relative risks, however, baseline rates for various age groups must be given serious consideration. Importance of other predisposing conditions mentioned above in determining relative and absolute risks has not as yet been quantified; quite likely the same synergistic action exists, but perhaps to a lesser extent.

RISK OF DOSE—In an analysis of data derived from several national adverse-reaction reporting systems, British investigators concluded that risk of thromboembolism, including coronary thrombosis, is directly related to dose of estrogen in OC's. Preparations containing 100 mcg or more of estrogen were associated with higher risk of thromboembolism than those containing 50-80 mcg. Their analysis did suggest, however, that quantity of estrogen may not be the sole factor involved. This linding has been confirmed in the U.S.

ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASES—A large prospective study carried out in the UK estimated the mortality rate per 100,000 women per year from diseases of the circulatory system for users and nonusers of OC's according to age, smoking habits, and duration of use. Overall excess death rate annually from circulatory diseases for OC users was estimated to be 20 per 100,000 (ages 15-34—5/100,000; ages 35-44—33/100,000; ages 44-9—140/100,000), risk being concentrated in older women, in those with long duration of use, and in cigarette smokers. It was not possible, however, to examine interrelationships of age, smoking, and duration of use, nor to compare effects of continuous vs. intermittent use. Although the study showed a 10-fold increase in death due to circulatory diseases in users for 5 or more years are 10-fold increase in death due to circulatory diseases in users for 5 or more years, all these deaths occurred in women 35 or older. Until larger numbers of women under 35 with continuous use for 5 or more years are available, it is not possible to assess magnitude of relative risk for this younger group. Available data from a variety of sources have been analyzed to estimate risk of death associated with various methods of contraception. Estimates of risk of death for each method include combined ske of contraceptive method (e.g., thromboembolic and thrombotic disease in the case of OC's) plus risk attributable to pregnancy or abortion in event of method failure. This latter risk varies with effectiveness of method. The study concluded that mortality associated with all methods of brith control is low and below that associated with childbrith. with the exception of study concluded that mortality associated with all methods of birth control is low and below that associated with childbirth, with the exception of OC's in women over 40 who smoke. Lowest mortality is associated with condom or diaphragm backed up by early abortion. Risk of thromboem-bolic and thrombotic disease associated with OC's increases with age after about 30 and, for MI, is further increased by hypertension, hyper-cholesterolemia, obesity, diabetes, or history of pre-eclamptic toxemia, and especially cigarette smoking. Physician and patient should be alert to earliest manifestations of thromboembolic and thrombotic disorders (e.g. thromboembolic more prepared in the proposition of the proposition of

and especially cigarette smoking. Physician and patient should be alert to earliest manifestations of thromboembolic and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis, and mesenteric thrombosis). Should any of these occur or be suspected, the drug should be discontinued immediately. A 4- to 6-fold increased risk of postsurgery thromboembolic complications has been reported in OC users. If feasible, OC's should be discontinued at least 4 weeks before surgery of a type associated with increased risk of thromboembolism or prolonged immobilization. PERSISTENCE OF RISK OF VASCULAR DISORDERS—Findings from one study in Britan involving cerebrovascular disease and another in the U.S. concerning MI suggest an increased risk of these conditions in users of OC's persists after discontinuation of the OC's. In the British study, risk of cerebrovascular disease remained elevated in former OC users for at least 6 years after discontinuation. In the U.S. study, increased risk of MI persisted for at least 9 years in women 40 to 49 years old who had used OC's for 5 or more years. Findings in both studies require confirmation since they are inconsistent with other published information. 2. Ocular Lesions—There have been reports of neuro-ocular lesions such as optic neurits or retinal thrombosis associated with use of OC's. Discontinue OC's if there is unexplained, sudden or gradual, partial or complete loss of vision, onset of proptosis or diplopia; papilledema; or retinal-vascular lesions, and institute appropriate diagnostic and therapeutic measures.

3. Carcinoma—Long-term continuous administration of either natural

or synthetic estrogen in certain animal species increases frequency of or synthetic estrogen in certain animal species increases frequency of carcinoma of the breast, cerviar, again, and liver. Certain synthetic progestogens, none currently untained in OC's, have been noted to increase incidence of mammary nodules, benign and malignant, in dogs, in humans, 3 case-control studies have reported an increased risk of endometrial carcinoma associated with prolonged use of exogenous estrogen in postmenopausal women. One publication reported on the first 2T cases submitted by physicians to a registry of cases of adenocarcinoma of the endometrium in women under 40 on OC's. Of cases found in women without predictions as the control of the endometrium in women under 40 on OC's. Of cases found in women without predictions as the control of t cinoma of the endometrium in women under 40 on OC's. Of cases found in women without predisposing risk factors (e.g., irregular bleeding at the time-OC's were first given, polycystic ovaries), nearly all occurred in women who had used a sequential OC. These are no longer marketed. No evidence has been reported sungasting increased risk of endometrial cancer in users of conventional combination or progestogen-only OC's. Several studies have found no increase in breast cancer in women taking OC's or estrogens. One study, however, while also noting no overall increased risk of breast cancer in women on OC's, found an excess risk in subgroups of OC users with documented beingin breast disease.

increased risk of breast cancerum women on OC's, found an excess risk in subgroups of OC users with documented being breast disease. Reduced occurrence of beingmbreast tumors in users of OC's has been well documented. In summary, there is at present no confirmed evidence from human studies of increased risk of cancer associated with OC's. Close clinical surveillance of all women on OC's is, nevertheless, essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be taken to rule out malignancy. Women with a strong family history of breast cancer or with breast nodules, fibrocystic disease, or abnormal mammograms should be monitored with particular care if they elect to use OC's.

4. Hepatic Tumors—Benign heisatic adenomas have been found to be associated with use of OC's. One study showed that OC's with high hormonal potency were associated with higher risk than lower potency OC's. Although benign, hepatic adenomas may rupture and may cause death through intra-abdominal hemorrhage. This has been reported in short-term as well as long-term users. I'wo studies relate risk with duration of use of OC's, the risk being mucin grater after 4 or more years' use. While hepatic adenoma is rare, it should be considered in women on OC's. Relationship of these drugs tot us-type of malignancy is not known.

5. Use in or Immediately Preceding Pregnancy, Birth Defects in Offspring, and Malignancy in Female Offspring—Use of female sex hormones—both estrogenic and progestational agents—during early pregnancy may seriously damage the offspring. It has been shown that females exposed in utero to diethylistilbestrol, armorsteroidal estrogen, have increased risk of developing in later life a formor vaginal or cervical cancer ordinarily extremely rare. This risk has been estimated to be of the order of 1 in 1,000 exposures or less. Although there is no evidence now that OC's further enhance risk of developing this type of malignancy, such patients should be monitor should be monitored with particular care if they elect to use OC's. Furthermore, 30 to 90% of such exposed women have been found to have eighthelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether this condition is a precursor of vaginal malignancy. Male children so exposed may develop abnormalises of the urogenital treat. Although similar data are not available with use of other estrogens, it cannot be presumed they would not induce similar changes. An increased risk of congenital anomalies, including heart defects and limb defects. has been reported with use of sex hormones, including OC's, in pregnancy. One case-control study estimated a 4.7-fold increase in risk of limb-eduction defects in infants exposed in utero to sex hormones (OC's, herromonal withdrawal tests for pregnancy, or attempted treatment for threatmend abortion). Some exposures involved only a few days. Datasuggest that risk of limb-reduction defects in exposed fetuses is somewhall-ess than 1 in 1,000 live births. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or havitual abortion. There is considerable evidence that estrogens are ineffective to these indications, and there is no evidence from well-controlledistudies that progestogens are effective. evidence that estrogens are ineffective for these indications, and there is no evidence from well-controllects tudies that progestogens are effective. There is some evidence that imploidy and possibly other types of polypolicy are increased among abortuses from women who become pregnant soon after ceasing OC's. Embryos with these anomalies are virtually always aborted spontaneously. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping OC's is unknown. It is recommended that, for any patient who has missed 2 consecutive periods, pregnancy should be ruled out before continuing OC's. If the patient has not adherent to the prescribed schedule, the possibility of pregnancy should be cognificated at time of the twisted the prescribed or prescribed. OC's If the patient has not adhered to the prescribed schedule; the possibility of pregnancy should be considered at time of first missed period, and further use of OC's should be withheld until pregnancy has been ruled out. If pregnancy is confirmed, the patient should be apprised of the potential risks to the fetus, andiadivisability of continuation of the pregnancy should be discussed. It is aslor recommended that women who discontinue OC's with intent of becoming pregnant use an alternate form of contraception for a period of time before attempting to conceive. Many clinicians recommend 3 months, although no precise information is available on which to base this. The administration of progestogen-estrogen combinations to induce withdrawed bleadings should not be used as a test. combinations to induce withdrawal bleeding should not be used as a test

combinations to induce withdrawel bleeding should not be used as a tes of pregnancy.

6. Galbladder Disease—Studies—eport increased risk of surgically confirmed galbladder disease in users of OC's and estrogens. In one study, increased risk appeared after 2 years' use and doubled after 4 or 5 years use. In one of the other studies, increased risk was apparent between 6 and 12 months' use.

7. Carbohydrate and Lipid Metabolic Effects—Decrease in glucose tolerance has been observed in a significant percentage of patients on OC's. For this reason, prediabetic andmanetic patients should be carefully observed while on OC's. Increase in the patients of that phospholing the on OC's increase in the properties of that phospholing the properties and the properties and that phospholing the properties and the properties are the properties and the properties and the properties and the properties are the properties and the properties and the properties are the properties are the properties are the properties and the properties are the properties a

observed while on OC's. Increase in triglycerides and total phospholipids has been observed in patients on OC's; clinical significance of this finding remains to be defined.

8. Elevated Blood Pressure—Increase in blood pressure has been reported in patients on OC's. In some women, hypertension may occur within a few months of beginning DC's. In the 1st year of use, prevalence of women with hypertension is low in users and may be no hipher than that of a comparable group of norusers. Prevalence in users increases, however, with longer exposure, and in the 5th year of use is 2½ to 3 times the reported prevalence in the 1st year. Age is also strongly correlated with development of hypertension in OC users. Women who previously have had hypertension during pregnancy may be more likely to develop elevation of blood pressure on OC's. Hypertension that develops as a result of taking OC's usually returns to normal after discontinuing the drug. 8. Elevated Blood Pressure—Increase in blood pressure has bee

as a result of taking OC's usually returns to normal after discontinuing the drug.

9. Headache—Onset or exacerbation of migraine or development of headache of a new pattern which is resurrent, persistent, or severe, requires discontinuation of OC's and evaluation of the cause.

10. Bleeding Irregularities—Breakthough bleeding, spotting, and amenorrhea are frequent reasons for patients discontinuing OC's. In breakthough bleeding, as in all casescol irregular vaginal bleeding, and included the continuent of the

in minimizing menstrual irregularity, should be done only if necessary In minimizing menstrual irregularity, should be done only if necessary, since this may increase risk of thromboembolic disease. Women with past history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may have a tendency to remain anovulatory or to become amenorrheic after discontinuing OC's. Women with these preexisting problems should be advised of this possibility and encouraged to use other methods. Post-use anovulation, possibly prolonged, may also occur, in women without previous repulsariaes.

also occur in women without previous irregularities.

11. Ectopic Pregnancy—Ectopic as well as intrauterine pregnancy may occur in contraceptive failures.

occur in contraceptive failures

12. Breast-feeding—OC's given in the postpartum period may interfere
with lactation and decrease quantity and quality of breast milk. Furthermore, a small fraction of the hormones in OC's has been identified in the
milk of mothers on OC's, effects, if any, on the breast-fed child have not
been determined. If feasible, defer OC's until infant has been weaned.

Precautions—GENERAL—1. A complete medical and family history
should be taken prior to initiation of OC's. Pretreatment and periodic
mysical examinations should include precial reference to blood pressure should be taken prior to initiation of OC's. Pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Pap smear and relevant laboratory tests. As a general rule OC's should not be prescribed for longer than 1 year without another physical examination.

2. Under influence of estrogen-progestogen preparations, preexisting uterine leiomyomata may increase in size.

3. Patients with history of psychic depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. Patients becoming significantly depressed with on OC's should degree. Patients becoming significantly depressed with on OC's should

degree. Patients becoming significantly depressed while on OC's should stop OC's and use an alternate method to try to determine whether the

stop UC's and use an alternate method to try to determine whether the symptom is drug-related.

4. OC's may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, asthma, or cardiac or renal insufficiency.

5. Patients with a nest history of injurding during pregnagory have an

 Patients with a past history of jaundice during pregnancy have ar increased risk of recurrence while on OC's. If jaundice develops, OC's should be discontinued.

should be discontinued.

6. Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution.

7. OC users may have disturbances in normal tryptophan metabolism which may result in a relative pyridoxine deficiency. Clinical significance is ndetermined. . Serum folate levels may be depressed by OC's. Since the pregnant

woman is predisposed to development of folate deficiency and incidence of foliate deficiency increases with increasing gestation, it is possible that if a woman becomes pregnant shortly after stopping OC's, she may have a greater chance of developing folate deficiency and complications attrib-

a greater chance of developing folate deficiency and complications attributed to this deficiency.

Information for the Patient—See Patient Package Labeling.

Laboratory Tests—1. The pathologist should be advised of OC therapy
when relevant specimens are submitted.

2. Certain endocrine- and liver-function tests and blood components may
be affected by estrogen-containing OC's:

Legender (with hempethaling) execution.

a. Increased sulfobromophthalein retention

a. Increased sulfobromophthalein retention.
b. Increased prothrombin and factors VII, VIII, IX, and X: decreased antithrombin 3; increased norepinephrine-induced platelet aggregability.
c. Increased thyroid-binding globulin (TBG) leading to increased circulating total-thyroid hormone, as measured by protein-bound iodine (PBI).
T4 by column, or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG; free T4 concentration is unaltered.
d. Decreased pregnanediol excretion.
e. Reduced response to metryagone test.

e. Reduced response to metyrapone test
Drug Interactions—Reduced efficacy and increased incidence of breakthrough bleeding have been associated with concomitant use of rifampin
A similar association has been suggested with barbiturates, phenyibutazone phenyton sodium, ampicillin and tetracycline.
Carcinogenesis, Mutagenesis, Impairment of Fertility—See Warnings
section #3, 4, and 5 for information on carcinogenesis, mutagenesis,
and impairment of fertility.
Pregnancy—Category X. See Contraindications, Warnings.
Werein Markers—See Warnings. Recause of the notential for adverse.

Pregnancy—Category X. See Contraindications, Warnings.

Nursing Mothers—See Warnings. Because of the potential for adverse reactions in nursing infants from oral contraceptive tablets, a decision should be made whether to discontinue the drug, taking into account the

should be made whether to discontinue the drug, taking into account the importance of the drug to the mother.

Adverse Reactions—An increased risk of these serious adverse reactions has been associated with use of OC's (see Warnings): thrombophlebits, pulmonary embolism, coronary thrombosis, cerebral thrombosis, cerebral hemorrhage, hypertension, gallbladder disease, benign hepatomas, congenital anomalies. There is evidence of an association between the following conditions and use of OC's although additional confirmatory studies are needed: mesenteric thrombosis, neuro-ocular lesions, e.g., retiral thrombosis and order pauritis.

studies are needed: mesenteric thrombosis, neuro-occular lesions, e.g., retinal thrombosis and optic neuritis.

The following adverse reactions have been reported in patients on OC's and are believed to be drug-related. Nausea and/or vomiting, usually the most common adverse reactions, occur in approximately 10 percent or less of patients during the first cycle. Other reactions, as a general rule, are seen much less frequently or only occasionally. Gastrointestinal symptoms (such as abdominal cramps and bloating), breakthrough bleeding, spotting, change in menstrual flow; dysmenorrhea, amenorrhea during and after treatment, temporary infertifity after discontinuance of treatment; edema; chloasma or melasma which may persist; breast changes; tenderness, enlargement, and secretion; change in weight (increase or decrease); change in cervical erosion and cervical secretion; possible diminution in lactation when given immediately postpartum; cholestatic jaundice; migraine; increase in size of uterine leiomyomata; rash (allergic); mental depression; reduced tolerance to carbohydrates; vaginal candidiasis; change in corneal curvature (steepening), intolerance to contact lenses. tact lenses.

The following adverse reactions have been reported in users of OC's, and The tollowing adverse reactions have been reported in users of U.S., and the association has been neither confirmed nor refutely premenstrual-like syndrome, cataracts, changes in libido, chorea, changes in appetite, cystitis-like syndrome, headache, nervousness, dizziness, hirsutism, loss of scalp hair, erythema multiforme, erythema nodosum, hemorrhagic eruption, vaginitis, porphyria.

scap hair, etymenia monitorine, etymenia noutosini, nemornagic eri tion, vaginitis, porphyria. Acute Overdose—Serious ill effects have not been reported following acute ingestion of large doses of OC's by young children. Overdosage may cause nausea, and withdrawal bleeding may occur in females.



each tablet contains 0.15 mg levonorgestrel and 0.03 mg ethinyl estradiol.

(LEVONORGESTREL AND ETHINYL ESTRADIOL TABLETS)

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CLINICAL SECTION

Clinical Opinion

TORCH tests and what they mean

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TORCH tests can be used to document immunity or recent infections. Many laboratories now offer TORCH tests and there are a large variety of methods, kits, and reporting systems. Some of the TORCH tests are not accurate and should be avoided. In general, we prefer enzyme immunoassays for documentation of past infections or immunity. To demonstrate recent infections with toxoplasmosis and rubella we use IgM enzyme-linked immunosorbent assay tests but for cytomegalovirus and herpes infections we prefer virus isolation. Physicians should send specimens only to laboratories that participate in proficiency surveys or similar tests that document the accuracy of the laboratory. All TORCH test sera should be held by the laboratory for 1 year to permit repeat testing and analysis with subsequent specimens if necessary. (AM J OBSTET GYNECOL 1985;152:495-8.)

Key words: Toxoplasmosis, cytomegalovirus, herpes, rubella, pregnancy

TORCH tests are now available from many hospital and commercial laboratories throughout the United States. It is estimated that for rubella alone more than 7 million tests are run each year. Among the approximately 1200 laboratories participating in the Center For Disease Control's Proficiency Testing Survey—1984, rubella antibody tests were performed by 491 laboratories, toxoplasma by 237, cytomegalovirus by 192, and herpes by 127. In addition, 68 laboratories conducted IgM rubella tests. Many smaller laboratories also forward their specimens to central laboratories for these tests.

With all of these tests in use there has been a growing business in kits for TORCH tests. Almost two dozen manufacturers are producing various TORCH kits that use a variety of laboratory methods.² Most kits are being marketed to larger laboratories, but some will soon be sold directly to physicians for "office laboratory" use.

The great abundance of tests and kits has resulted in several problems: (1) Multiple methods—There are now nine different laboratory methods in general use for rubella antibody determinations and additional methods for the other TORCH tests. Not only do these methods give different results but some are reported in units that are difficult to relate to standard dilution titers. (2) Accuracy—There is often variation in TORCH titers. Even the best laboratories have some variability and considerably greater differences occur between laboratories. Physicians must be aware that some TORCH results may be in error. (3) Test selection—Physicians must select the best and most cost-effective test for the clinical problem under consideration. This requires knowledge about the various TORCH tests and what they mean.

In this article the TORCH tests are reviewed in relation to methods, accuracy, and test selection. Specific tests are recommended for determining immunity and for documenting recent infections.

Multiple methods

The major methods that are currently used for TORCH tests are shown in Table I. At least four methods are now in general use for each TORCH agent, and for rubella nine methods are being performed. For most methods, several different commercial kits are available.² In addition, a number of laboratories prepare their own reagents for these tests.

The large number of test methods has resulted in a variety of types of reporting. Some test results are given

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Table I. TORCH test methods: Usage and accuracy

Agent and test method	Test usage (% of total)	Centers for Disease Control qualitative test performance*	
Toxoplasma		+	
Indirect immunofluorescence (IIF)	78.5	97.0	0.0
Passive indirect hemagglutination (PHA)	10.8	100.0	0.0
Fluoroimmunoassay (FIA)	7.6	100.0	6.2
Enzyme immunoassay (EIA-ELISA)	3.1		0.0
Rubella		+	-
Latex agglutination—slide	26.4	100.0	0.9
Enzyme immunoassay (EIA-ELISA)	23.4	100.0	0.0
Passive hemagglutination (PHA)	16.7	98.6	1.3
Hemagglutination inhibition (heparin/dextran)	14.7	100.0	71.8
Fluoroimmunoassay (FIA)	6.9	100.0	0.0
Hemagglutination inhibition—kaolin	5.3	95.0	0.0
Indirect immunofluorescence (IIF)	2.7	100.0	0.0
Radioimmunoassay (RIA)	2.5	100.0	0.0
Reverse passive latex agglutination (RPLA)	1.4		0.0
Cytomegalovirus		+	+
Indirect immunofluorescence (IIF)	45.9	98.5	93.6
Complement fixation (CF)	41.8	100.0	100.0
Enzyme immunoassay (EIA-ELISA)	7.0	90.0	83.3
Fluoroimmunoassay (FIA)	5.3	100.0	100.0
Herpesvirus		+	+
Complement fixation (CF)	62.2	47.2	75.0
Indirect immunofluorescence (IIF)	18.5	95.4	100.0
Fluoroimmunoassay (FIA)	12.2	100.0	100.0
Enzyme immunoassay (EIA-ELISA)	6.7	75.0	

^{*}Adapted from Centers for Disease Control Proficiency Testing Survey—1984.¹ Coded sera shown as + or -. Qualitative results from test laboratories shown as percent positive.

only as positive or negative while others are reported as "quantitative" results. Some of the "quantitative" tests are still given as titers based on the last serial two-fold dilution of serum showing a reaction. Many of the newer tests, however, use other dilution steps, report results as optical density or other units, or relate the titer to that of a reference serum.

Because of the large variety of methods, kits, and reporting systems it is important for the physician to send all serial specimens to the same laboratory for the same test. If significant differences are found in the results, then all specimens should be rerun in the same test at the same time. The physician should also become familiar with the normal values and ranges for each of the tests being performed by the laboratory.

The relative usage of the various TORCH methods at the Centers for Disease Control test laboratories is shown in Table I. For toxoplasmosis, the indirect immunofluorescence method is now used by the greatest number of laboratories. This test is relatively easy to perform. With rubella, the very simple latex agglutination test has become the most popular and the highly sensitive enzyme-linked immunosorbent assay method is also used frequently. The traditional hemagglutination inhibition test, however, has been decreasing in use for several years. Cytomegalovirus tests are now usually performed by the relatively simple indirect immunofluorescence or complement fixation methods. Herpestests that report no subtype are most often run by com-

plement fixation and the subtypes 1 and 2 with indirect immunofluorescence.

Accuracy

Some of the considerations that influence the selection of a test method by a laboratory are simplicity, speed, and cost. The most important factor, however, is accuracy. The 1984 Centers for Disease Control results for two coded sera tested by the participating laboratories are given in Table I.1 In general, the data for 10 to 100 laboratories are given for each method, depending on the percentage of laboratories using that method. It should be noted that the data shown are only for qualitative results with two test sera. Further data as well as quantitative findings are available in the full report and in similar reports from previous years. 1, 3, 4 Also, the Centers for Disease Control participating laboratories are among the largest and best known in the country. Thus the results do not necessarily apply to all laboratories that offer these tests.

One fundamental conclusion can be made concerning accuracy. Even for qualitative tests done by these generally larger laboratories, some clinically important laboratory errors were present. For example, with toxoplasmosis, 6.2% of the fluoroimmunoassay tests were false positive. More important, for rubella, 71.8% of the hemagglutination inhibition (heparin/dextran) tests were false positive. Problems with the removal of inhibitors in some sera tested by hemagglutination in-

hibition are well known but continue to cause false positive results in some laboratories. 1.3 A method of identifying persistent inhibitors in the hemagglutination inhibitor test has been described in detail. Evaluation of the accuracy of the cytomegalovirus and herpes tests was limited because both test sera were positive. For cytomegalovirus, however, with indirect immunofluorescence and enzyme-linked immunosorbent assay tests, the specimens were incorrectly reported as negative by up to 16% of the laboratories. With herpes, very significant errors were noted, particularly with the most popular complement fixation test where as much as 52% of results were in error.

Clearly, the physician must recognize that some TORCH titers are in error and results such as those reported in Table I for certain rubella hemagglutination inhibition and herpes complement fixation tests are worthless. Unfortunately, it may be difficult for the physician to determine the accuracy of the laboratory that he uses. First, some laboratories do not participate in tests of coded specimens. Second, the Centers for Disease Control, the College of American Pathologists, and the American Society of Clinical Pathologists do not publish the proficiency results for the individual laboratories that participate in their tests. At present, the only recourse available to the physician is to require that all specimens for TORCH tests be saved frozen by the laboratory for 1 year. With those specimens, repeat testing can be ordered for all results that are inconsistent with prior laboratory results or clinical findings. The specimens can also be sent to a reference laboratory for further testing and, if subsequent sera are tested, they can be run in the same test with the earlier specimen. We recommend that physicians send specimens only to laboratories that participate in the Centers for Disease Control TORCH proficiency tests and/or similar survey series for selected TORCH agents such as those offered by the College of American Pathologists or the American Society of Clinical Pathologists. Also, physicians or physician groups should make regular reviews of their laboratory's proficiency results for each of the TORCH agents to verify the current accuracy of the laboratory and to encourage the utilization of the best methods possible.

Test selection

TORCH tests are used clinically for two main purposes: (1) demonstration of past infection or immunity and (2) documentation of recent infection. For each purpose, certain tests methods can be selected on the basis of simplicity, speed, cost, and accuracy. The tests that we use in our laboratory are shown in Tables II and III.

Past infection. To demonstrate past infection or immunity, the test must be directed at detecting IgG an-

Table II. Tests recommended for documentation of past infection or immunity*

Test method
Passive indirect hemagglutination (PHA)
Enzyme immunoassay (EIA-ELISA) Latex agglutination—slide Enzyme immunoassay (EIA-ELISA)
Hemagglutination inhibition—kaolin Passive indirect hemagglutination (PHA)
Enzyme immunoassay (EIA-ELISA) Passive indirect hemagglutination (PHA)

*With all TORCH infections, immunity may not be complete. Recurrent infections with cytomegalovirus and herpes are frequent. Herpes tests are not specific for types 1 and 2.

tibody. The tests shown in Table II are quite sensitive and specific. The presence of antibody (a positive test) can be taken as evidence of past infection; however, it must be remembered that antibody does not necessarily mean complete immunity. For toxoplasmosis, patients may occasionally have exacerbations of the illness, such as toxoplasmosis in the eye, in the presence of prior antibody. With rubella, there are very rare reports of individuals with second infections. Also, both cytomegalovirus and herpes infections can recur in patients who had prior infections and antibody. In fact, recurrent genital herpes (type 2) is a major problem with that disease.

For pregnant women, TORCH tests can be of particular value. Antibody to toxoplasmosis and rubella can be taken as evidence of past infection. The presence of antibody indicates that there is essentially no risk for problems with these infections in future pregnancies. With cytomegalovirus, the presence of antibody indicates past infection. Subsequent children may be infected with cytomegalovirus at birth but the risk for damage is very low. The cytomegalovirus tests shown in Table II are quite specific. Antibody tests to herpes, however, are not specific and are of relatively little clinical value. Most laboratories provide only "generic" herpes tests that are not specific for type 1 or type 2. Thus a positive test may be due to a herpes type 1 infection of the lips that occurred during childhood. This would be indistinguishable from antibody following a recent genital infection with herpes type 2. Some laboratories report their tests as herpes type 1 or 2 because the virus antigen they use is type 1 or 2. However, this does not mean that the antibody test results are specific for type 1 or 2. Very few laboratories can perform the special tests that are actually specific for types 1 and 2, and these are usually run as research investigations.

Table III. IgM tests for demonstrating recent infections

Agent	Age group	Usuml results	Infrequent results
Toxoplasmosis	Adult	Pos. at diagnosis; significant titer for 4 mo (FA) to 8 mo (ELISA) Lower titer for ≥1 yr	Significant titer for ≥1 yr
	Child	Pos. at birth: lasts 6-12 mo	
Rubella	Adult	Pos. after 1 wk; lasts 1 mo	Rarely lasts 1-2 yr; some pos. with vaccine
	Child	Pos. at birth: lasts 6-12 mo	
Cytomegalovirus*	Adult	Primary pos. at diagnosis and for 4-8 mo (ELISA)	Some tests cross with Epstein-Barr virus, varicella zoster, and rheumatoid factor; rare pos. with reactivation of infection
	Child	50% pos. with FA; 90% pos. with ELISA or RIA	
Herpes*	Adult	Primary pos. for 1-6 mo	
**	Child	Late pos.	

^{*}For cytomegalovirus and herpes, infection is usually best demonstrated by virus isolation.

Recent infection. Recent TORCH infections can be documented by showing seroconversions with the use of the same tests listed in Table II. This requires paired sera bracketing the infection and in most cases only a single convalescent serum is available. Fortunately, IgM tests that can be used for this purpose are now available. IgM-specific antibody usually develops shortly after the onset of the infection and persists for 1 or more months, as shown in Table III.

IgM tests are most useful for toxoplasmosis and mubella. With recent toxoplasmosis, significantly elevated titers are usually present for 4 months (fluoroimmunoassay test) to 8 months (enzyme-linked immunoserbent assay test). For rubella, the tests become positive 1 week after the rash and persist for 1 month. The variations with the tests and findings with children are also shown in Table III. IgM tests for cytomegalovirus and herpes are available in some laboratories. Cytomegalovirus tests have some problems with false negative results, cross reactions with other related viruses, and positive tests with recurrent cytomegalovirus. Herpes IgM tests are positive with primary infections. New type-specific herpes IgM tests are becoming available. For both cytomegalovirus and herpes, the diagnosis is usually best made by virus isolation.

Comment and conclusions

TORCH tests are available from many hospital and commercial laboratories. A variety of methods are used and reports are given in various units. Because of the differences between methods and reporting units, physicians should send serial specimens to the same laboratory. It is necessary to be familiar with the normal values and ranges for the laboratory used.

The accuracy of TORCH tests is variable. For example, the Centers for Disease Control 1984 data showed as much as 71.8% false positive tests with ru-

bella hemagglutination inhibition by the heparin/dextran adsorption method. Herpes complement fixation tests were also very unreliable. The physician must recognize that some TORCH titers are in error. All specimens for TORCH tests should be saved frozen by the physician or by the laboratory for 1 year so that repeat testing can be performed. Also physicians or physician groups should regularly review the proficiency test results for the laboratories they use.

Tests must be selected to (1) demonstrate past infection or immunity and (2) document recent infection. We have identified the tests that we use for these purposes (Tables II and III). We recommend routine tests for rubella antibody for women of childbearing age and immunization of women who do not have antibody. We only perform toxoplasmosis testing for patients with clinical findings suggestive of the disease or histories of exposure to infection. IgM tests are of value for demonstrating recent infection with toxoplasmosis and rubella. We generally prefer to use virus isolation for identifying current cytomegalovirus and herpes infections.

We are grateful for the use of the excellent Proficiency Testing Summaries prepared by Drs. Taylor and Przybyszewski of the Centers For Disease Control, Atlanta, Georgia.

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Clinical Articles

Obstetric outcome in women with epilepsy

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A comparison of 150 pregnancies in women with epilepsy and 150 pregnancies in matched nonepileptic control women showed similar rates of pregnancy-induced hypertension, albuminuria, premature contractions, premature labor, and bleeding in pregnancy. Duration of labor, blood loss at delivery, cesarean section rates, and vacuum extraction rates were also similar among epileptic and control groups. There were five perinatal deaths in the epileptic group and two in the control group. A fetal heart rate tracing during a material grand mal seizure showed bradycardia, reduced short-term and long-term variability, and late decelerations suggesting asphyxia. It is concluded that grand mal seizures during pregnancy should be avoided by the use of antiepileptic drugs. Women with epilepsy require antenatal neurological and obstetric follow-up during pregnancy. (AM J OBSTET GYNECOL 1985;152:499-504.)

Key words: Epilepsy, anticonvulsants, pregnancy, cesarean section, perinatal mortality

During pregnancy and labor, women with epilepsy reportedly have a slightly increased risk of bleeding, 1, 2 toxemia, 1 prematurity, 1, 3 fetal asphyxia, 1, 4 and perinatal death. 1, 5-7 They also appear to have an increased rate of cesarean sections 1 and vacuum or forceps extractions. 1 The reason for the observed excess of obstetric complications and interventions among women with epilepsy is not quite clear. It is not known whether seizures or effects of antiepileptic drugs are involved. Series with no increase in obstetric complications or interventions in women with epilepsy have also been reported. 8, 9

We present here the results of a prospective study on the course and outcome of pregnancies in women with epilepsy and outline some guidelines for their management.

Patients and methods

One hundred thirty-nine epileptic women had 150 pregnancies (0.58% of total deliveries) of more than 24 weeks' duration and gave birth to 152 infants in the 4-year period from 1976 to 1979. All patients had had at least two epileptic seizures and fulfilled the criteria

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Table I. Seizure types in 139 epileptic women

Seizure type	No. of women		
Grand mal (tonic and clonic convulsive)	88		
Psychomotor (temporal lobe)	16		
Both grand mal and psychomotor	19		
Focal motor or sensory Other or unclassified	10		
Total	139		

All seizures during lifetime were counted for classification.

of the World Health Organization Dictionary of Epilepsy. ¹⁰ Seizure types of the mothers are shown in Table I. Antiepileptic treatment and the drug levels in the maternal serum during the third trimester are presented in Table II.

Patients were seen monthly during the first and second trimesters at the outpatient clinic for clinical and ultrasonic examination and assessment of antiepileptic drug levels in the maternal serum. In the last trimester, fetal well-being was monitored by nonstressed fetal heart rate (FHR) testing. The patients were seen fortnightly after 32 weeks and weekly after 36 weeks.

The dosage of antiepileptic drugs was increased during one third of the pregnancies because of increased occurrence of seizures. In the remaining two thirds, the previous dosage was maintained throughout pregnancy; no dose reductions were made but in 11 patients the number of drugs used was reduced. Drug levels were kept low in order to keep their eventual effects upon the fetus as small as possible. Levels lower than

	Drug given singly (n) Drug given in combination Total users* (n) (n)	Drug given in	Tables	Serum level (mg/L)	
Drug			Median	Range	
Phenytoin	59	45	104	4.5	0.3-18
Carbamazepine	21	21	42	5.0	1.2-11
Phenobarbital	0	26	26	7.2	0.5-23
Primidone	1	4	5	4.8	2.4-6.8
Valproic acid	1	8	9	24	19-51
Clonazepam	0	5	5		-
Ethosuximide	0	2	2		
Diazepam	0	2	2	<u> </u>	
No drugs		_	- 18		_

Table II. Antiepileptic drugs and their serum levels in third trimester of pregnancy

Levels are medians for thirty-third to fourtieth week of pregnancy.

Table III. Social class of epileptic parturients and next women giving birth (unmatched controls), based on woman's occupation*:†

Social class	Description	Epileptic women (No.)	Next parturients (No.)
I	Academic degree, etc.	9	31
II	Nonacademic "white collar"	42	51
III	Skilled manual laborer	68	57
IV	Unskilled manual laborer	31	11
Total		150	150

 $[\]chi^2 = 19.5$, df = 3, p < 0.001.

the so-called "therapeutic" drug levels were usually accepted if epilepsy remained well controlled.

Two sets of control groups (unmatched and matched) were used as follows.

In order to examine differences between epileptic women and the general parturient population with regard to age, parity, and social class, each parturient with epilepsy was compared with the next woman giving birth (unmatched control).

Matched control subjects were selected as follows. After delivery of the infant of each epileptic woman, the next pair (mother and child) who fulfilled each of the following criteria was identified: Mother's age within ±3 years, same parity, same social class, and same fetal sex. Social class was determined by the woman's profession, with the standard four-step classification of the Helsinki City Bureau of Statistics. Comparisons of obstetric and fetal outcomes were made between the epileptic and matched control groups. The race of all subjects was Caucasian.

Epilepsy per se was not considered an indication for induction of labor. During labor and delivery, the epileptic women were treated according to the general principles of this hospital. FHR tracings during labor were obtained in 110 epileptic women and in 118 matched control subjects, and fetal scalp blood samples

for suspected asphyxia in 20 epileptic women and in 21 matched control subjects.

Perinatal deaths included stillborn fetuses weighing >500 gm and neonatal deaths during the first week of life.

Statistical methods included the unpaired t test, Mann-Whitney U test, exact probability calculation, and χ^2 test. Values of p < 0.05 were considered as statistically significant. Relative risk and its 95% confidence limits were calculated for each pregnancy complication.

Results

The social class of epileptic parturients was clearly lower than that of unmatched control subjects (Table III). The mean age at delivery $(27.1 \pm 4.7 \text{ years}, \text{mean} \pm \text{SD})$ and parity (65% with first delivery) of women with epilepsy were similar to those of unmatched control subjects $(28.0 \pm 4.4, 56\% \text{ with first delivery})$. No differences between epileptic women and the unmatched or matched control subjects were observed with regard to maternal stature, prepregnancy weight, or weight gain during pregnancy.

The percentage of smokers was 24% among the epileptic group and 27% among the matched control group (NS). Gestational length (mean \pm SD) was 276 \pm 13 days for epileptic women and 277 \pm 12 for

^{*}N = 150.

^{*}When housewife, husband's occupation counted.

[†]Standard classification of Helsinki City Bureau of Statistics.

Table IV. Complications of 150 pregnancies of women with epillepsy and 150 pregnancies of matched control women

	No. of complications		Relative risk	
Complication	Epileptic group	Gontrol group	Point estimate	95% Confidence limits
Arterial hypertension				
Essential*	3	3	1.0	0.2-5.2
Pregnancy-induced†	11	. 16	0.7	0.3-1.6
Proteinuria				
With hypertension	5	6	0.8	0.2-2.9
Without hypertension	1	2	0.5	0.04-5.9
Hemorrhage				4
Abruptio placentae	1	2	0.5	0.04-5.9
Other	14	15	0.9	0.4-2.0
Anemia (hemoglobin <105 gm/L)	5	4	1.3	0.3-4.9
Breech presentation	8	7	1.1	0.4-3.3
Twin pregnancy	2	1	2.0	0.2-23
Premature contractions requiring medication	15	22	0.7	0.3-1.4
Premature live birth (<37 completed weeks)	10	. 11	0.9	0.4-2.2
Urinary tract infection	12	7	1.7	0.6-4.6
Respiratory tract infection treated by antibiotics	21	16	1.3	0.7-2.6
Chemical diabetes	2	2	1.0	0.1-7.5
Hepatosis of pregnancy	1	2	0.5	0.04-5.9

Differences between epileptic and control groups were not statistically significant.

Table V. Obstetric interventions in epileptic women (N = 150) and matched control subjects (N = 150)

		Epileptic group	Control group	
Amnioce	entesis	9	-6	
Shirodka	ar operation	2	3	
	amniotomy	22	21	
Cesarear	i section	28	29	
Epidural	anesthesia	10	10	
Vacuum	extraction	7	8	
Forceps	extraction	1	0	
Manual	detachment of	6	3	
the pla or abr	acenta and/ asion			

Differences not significant.

matched control subjects (NS). The mean birth weight (mean \pm SD) of the infants was 3392 \pm 496 gm for the epileptic group and 3430 \pm 488 for the matched control group (NS).

Pregnancy complications occurred among epileptic women as frequently as among the matched control group (Table IV). Inductions, vacuum or forceps extractions, and cesarean sections were performed at equal rates for epileptic and matched control women (Table V). Epilepsy was the principal indication for four of the cesarean sections and an additional indication

Table VI. Findings in FHR tracings during labor of women with epilepsy (N = 110) and matched control subjects (N = 118)*

	No. of patients		
Finding	Epileptics	Controls	
<5 accelerations of >15 bpm	31	27	
>2 early decelerations	30†	16†	
>2 variable decelerations	26	16	
>2 late decelerations	13	12	
Tachycardia (basal heart rate >160 bpm during 10 min period)	2	5	
Bradycardia (basal heart rate <120 during 10 min period)	23	16	
Reduced baseline variability (<10 bpm during 30 min period)	2	4	

^{*}Stillbirths and elective cesarean sections were excluded. $\dagger \chi^2 = 4.6$, df = 1, p < 0.05.

for another three. Findings in FHR tracings during labor (Table VI) were similar in epileptic and matched control groups except that early decelerations were seen more often among epileptic women (p < 0.05). No differences between epileptic and matched control subjects were observed either in duration of labor or

^{*}Hypertension present before 24 weeks of gestation.

[†]Hypertension only after 24 weeks of gestation; systolic pressure ≥140 mm Hg and diastolic pressure ≥90 mm Hg on 2 separate days or 150/100 on 1 day. Only values after 30 minutes of rest were counted.

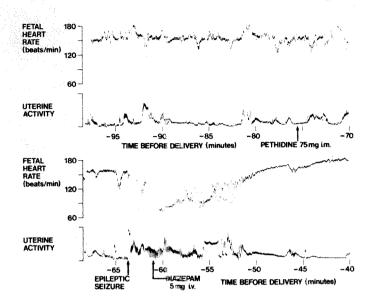


Fig. 1. FHR before, during, and after a maternal grand mal seizure during labor.

Table VII. Duration of labor and bleeding at vaginal delivery of 122 epileptic women and 121 matched control subjects

	Epileptic group		Control group	
	Median	Range	Median	Range
Duration of labor First stage (hr) Second stage (min) Third stage (min) Bleeding (L)	6.3 14 8 0.3	0.8-24 1-151 3-58 0-1.9	6.6 15 8 0.3	0.9-55 2-74 2-73 0-4.0

Differences not significant.

in the amount of bleeding at delivery (Table VII).

None of the 170 grand mal epileptic seizures (in 48 pregnancies) was followed within 24 hours by vaginal bleeding, rupture of amniotic membranes, initiation of uterine contractions, intrauterine death, or other recognizable obstetric complications. In the remaining 102 pregnancies there were no grand mal seizures.

Two women had a grand mal seizure during labor; both were delivered by cesarean section because of postictal changes in FHR and maternal sleepiness. Other types of seizure occurred during the labor of four other patients who were all delivered vaginally.

Fig. 1 shows the FHR traced with a scalp electrode before, during, and after a maternal grand mal seizure during labor. The cervix was then dilated 5 cm. The seizure lasted 3 minutes and was followed by a long wave of fetal bradycardia, reduced short-term and long-term variability, and a few decelerations. Sixty minutes after the seizure, a cesarean section was performed because, of suspected fetal asphyxia and maternal postictal lack of cooperation. The male infant

received an Apgar score of 8 at the age of 1 minute. The recovery of the mother and child was uneventful.

An Apgar score of <7 was given to four live-born infants of epileptic mothers and to infants of seven matched control subjects (NS). Malformations or minor anomalies were observed in 17 of 152 infants of epileptic mothers and in 14 of 151 infants of matched control subjects (NS).

There were three stillbirths at twenty-eighth, thirty-sixth, and thirty-eighth week and two deaths in the first week of life among the epileptic group; there were two stillbirths and no deaths in the first week among the matched control group (NS). None of the five epileptic mothers with a perinatal infant death had seizures during pregnancy, and the types and serum levels of their antiepileptic drugs were similar to those of 'e rest of the series (Table VIII).

Comment

In accordance with the results of Watson and Spellacy⁸ and Martin,⁹ the epileptic mothers of the present series did not have more pregnancy and labor complications than control subjects. A report from the Birth Registry of Norway² suggests that frequency of bleeding is significantly increased (1.7-fold) in pregnancies and deliveries of women with epilepsy; the specific rate of abruptio placentae was also raised (twofold). The Norwegian investigators have, however, pointed out the possibility of a bias in the reporting of bleeding, especially before labor.¹

Although increased rates of cesarean sections have been reported for epileptic women, ¹¹ there is no evidence that this would result from obstetric complications. Obstetricians may resort to a surgical delivery of

Table VIII. Perinatal deaths among 152 children born to epileptic mothers

Outcome	Gender	Drugs and last serum levels in pregnancy (mg/L)	Birth weight (gm)	Pregnancy complication	Necropsy finding
Stillbirth	F	Phenytoin, 4.3 Phenobarbital, 4.8	600	Preeclampsia	Horseshoe kidney
Stillbirth	M	Phenytoin, only first trimester	1500	None	Maceration
Stillbirth	F	Phenytoin, 7.1	3120	None	Maceration
Neonatal death	F	Phenytoin, 6.4	1270	Premature labor	Hyaline membrane disease
Neonatal death	M	Phenytoin, 5.3 Carbamazepine, 2.4	3100	Abruptio placentae	Abruptio placentae, asphyxia

the epileptic mother because of insecurity in handling such a case.¹² The present study suggests that epileptic women have an obstetric performance equal to that of other women and that there is no need for more than the usual rate of cesarean sections or vacuum (forceps) extractions for obstetric reasons, at least when the mothers have been under proper antenatal neurological and obstetric care.

A grand mal seizure during labor is rare but can cause transient fetal asphyxia.¹³ This is further confirmed by the case presented here. After a grand mal seizure in labor, a prompt cesarean section must be considered because of fetal risks and reduced maternal postictal cooperation. In our opinion, epilepsy can be considered as grounds for a cesarean section when (1) a grand mal seizure occurs during labor, (2) epilepsy is accompanied by a substantial neurological or mental deficit that might reduce the patient's cooperation during a vaginal delivery, (3) there is very poor seizure control in late pregnancy in spite of the treatment, for instance, daily psychomotor or weekly grand mal seizures, or (4) there is prior knowledge of the occurrence of severe seizures during heavy physical or mental stress

A slight increase in the frequency of fetal malformations has been observed in several studies on pregnancies of women with epilepsy. ¹⁴ Although no increase in malformations was evident in the present study, it was too small for a proper assessment of teratogenic risks. In addition to gross malformations, other fetal effects of seizures and/or drugs, such as impaired growth or function, should be considered. We have earlier reported on a smaller than expected head circumference of the children exposed in utero to carbamazepine or phenobarbital in the present series. ¹⁵

The perinatal deaths in the present study were not associated with maternal seizures or high drug levels in pregnancy. Although their number among epileptic women (n = 5) did not significantly differ from that among matched control subjects (n = 2), these figures conform to results of several previous reports on an

increased (twofold to threefold) risk of perinatal death for the offspring of women with epilepsy.^{1, 2, 4-7}

The rate of and reasons for perinatal deaths of children of epileptic mothers need to be further investigated.

Plasma concentrations of antiepileptic drugs tend to fall during pregnancy.16 In many cases this is not associated with an increase in the seizure frequency, and the patients can remain on a regimen of their previous dose.16 Our experience is that drug levels lower than the so-called "therapeutic" levels can be accepted when epilepsy remains well controlled. If there is an increase in the seizure frequency during pregnancy, the dose of the antiepileptic drug(s) should be increased. 16 This was the case in one third of the present series. Monitoring of the serum concentrations of antiepileptic drugs should be continued for the first 2 or 3 months of the puerperium.¹⁶ The doses, if increased in pregnancy, should be returned to their prepregnancy levels within 3 months after delivery in order to avoid intoxication. 16 Use of a single antiepileptic drug is currently preferred over use of multiple drugs.14 Women with epilepsy need neurological and obstetric follow-up during pregnancy. Most young women with well-controlled epilepsy who are receiving a long-term antiepileptic medication can be assured of a good maternal and fetal outcome. More studies are still needed, however, to assess the possible long-term effects of maternal epileptic seizures and antiepileptic drugs upon the child.

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Superficial laser vulvectomy

III. A new surgical technique for appendage-conserving ablation of refractory condylomas and vulvar intraepithelial neoplasia

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Despite the unique properties of the carbon dioxide laser, many surgeons do not know how to exploit the full potential of this sophisticated instrument. Effective laser operation on the vulva depends upon the accuracy of delineation of disease, the use of optimum power densities, and the ability to exercise precise control over depth of ablation. This article describes a surgical technique that capitalizes upon these principles, thereby maximizing the margin between favorable and poor outcomes. Superficial laser vulvectomy is a safe and efficient procedure in the hands of expert physicians, but should not be attempted by those who are less experienced. Indications for this operation and safeguards against surgical misadventure are also discussed. (AM J OBSTET GYNECOL 1985;152:504-9.)

Key words: Laser, vulvectomy, condyloma, vulvar intraepithelial neoplasia, surgical technique

Over the last decade, the prevalence of vulvar intraepithelial neoplasia in young women has increased dramatically, particularly the multifocal "Bowenoid" variety. Although the treatment originally proposed for squamous carcinoma in situ of the vulva was wide local excision, fears that the disease was preinvasive led to the widespread use of vulvectomy (simple or skinning) in such patients. However, most documented instances of invasion have occurred in immunosup-

pressed or elderly women. In healthy patients, the risk of malignant progression is insufficient to justify such a mutilating surgical procedure. Treatment of vulvar intraepithelial neoplasia is controversial, with recommendations ranging from wide excision to skinning vulvectomy. Wide excision of small foci produces excellent results, but multifocal or extensive lesions are difficult to treat by this method. In the past, the only other alternative was skinning vulvectomy with grafting. Although it was a definite improvement over conventional vulvectomy, cosmetic and functional results were still poor. Fortunately, the carbon dioxide laser offers an escape from this dilemma (Fig. 1), by providing an effective but nonmutilating treatment for this distressing, possibly premalignant disease.

As a consequence of changing social values, Western

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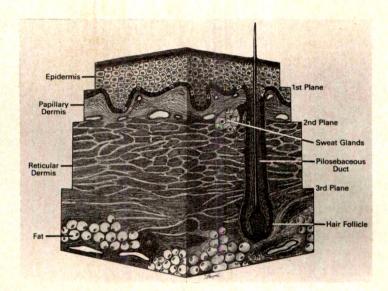


Fig. 1. A diagram depicting the first three surgical planes. Reading from surface to base, the points of reference for each plane are indicated as stepwise expansions. The first surgical plane corresponds to the basement membrane, the second to the papillary dermis, and the third to the midreticular dermis.

Table I. Summary of salient features of the four surgical planes that can be used for more accurate control of depth during carbon dioxide laser operations

	Surgical plane				
Parameter	First	Second	Third	Fourth*	
Target tissue	Surface epithelium	Dermal papillae	Pilosebaceous ducts	Pilosebaceous glands	
Zone of vaporization	Proliferating layer	Superficial papil- lary dermis	Upper reticular dermis	Midreticular dermis	
Zone of necrosis	Basement membrane	Deep papillary	Midreticular	Deep reticular	
Type of healing	Rapid, cosmetic	Rapid, cosmetic	Usually cosmetic, may hypertrophy	Atrophic or hyper- trophic; needs grafting	
Visual landmark	Opalescent cell debris	Scorched basement membrane	Coarse collagen fibers	"Sand grains" (skin appendages)	

^{*}Described in Reference 7.

society has also experienced an epidemic of sexually transmitted papillomaviral infections.8 Condylomas result from the direct transmission of human papillomaviral infections during physical contact. After an incubation period of 1 to 6 months, infected tissues undergo a phase of rapid epithelial and capillary proliferation. Even without treatment, many lesions will regress spontaneously over the succeeding 6 to 12 months, apparently as a result of a combined cellular and humoral immune response.9 Subsequent to simple therapy with caustic agents or thermal cautery, most patients with vulvar condylomas will be free of clinical lesions within 1 year. Since these infections are always more widespread than is appreciated by examination with the naked eye, it is difficult to explain the apparent success of such crude measures. However, the small residue of patients with unusually extensive lesions or

infections that are refractory to conventional treatments constitutes management problems, and is a source of frustration to patients and physicians alike. Several authors have reported favorably on the result of laser operations in such patients.10

Despite the unique physical properties of the carbon dioxide laser, using this instrument as a "spot welder" does not offer a solution to the treatment of patients who have refractory surrounding subclinical infection.6 Likewise, although it is a very simple surgical exercise to use a laser on a patch of vulvar intraepithelial neoplasia, poor control of depth can lead to full-thickness epithelial destruction and a cosmetic result that is no better than would follow any other type of third-degree burn. Hence, surgeons who use the carbon dioxide laser for the ablation of large areas of vulvar epithelium must learn to recognize the three surgical planes de-



Fig. 2. A broad sessile condyloma in the right upper portion of the field has been undercut to a deep dermal plane by using the laser as an excisional rather than an ablative tool. After the thick layer of charred proteins that ordinarily covers a laser impact site has been wiped away, the coarse collagen fibers of the deep dermis are readily visible. Surrounding condylomas have been vaporized.

fined in a previous article⁷ (Table I). Otherwise, laser operations will carry unacceptable risks of delayed healing and scar formation.^{5, 12} This article, the third in a series of five, describes the actual surgical technique of superficial laser vulvectomy.

Surgical technique

Preparation of patient. If a patient has condylomas that are sufficiently extensive or refractory to require treatment by this method, the area of affected epithelium will almost always be too large for treatment under local infiltration. Likewise, since vulvar intraepithelial neoplasia in young women is usually multicentric, and is often surrounded by even larger areas of subclinical papillomaviral infection, most such cases will also require general or regional anesthesia.

After the induction of anesthesia, the perineum is carefully shaved (both to facilitate colposcopy and to simplify postoperative care). The use of antiseptic solutions is neither desirable (because it impairs response of tissue to acetic acid) nor necessary (because of the high temperatures attained at the site of laser impact). Rather, the vulva and anus are soaked for 3 minutes with acetic acid or white vinegar (with the use of very wet cotton balls or gauze squares). The perineum is then carefully examined with a colposcope. The borders of any foci of vulvar intraepithelial neoplasia and the outer margins of the surrounding subclinical pap-



Fig. 3. The initial vaporization of benign condylomas, sufficient to extend the central crater to about the level of the surrounding epidermis. This maneuver will loosen the epidermal cells in the base of the condyloma, so that wiping with a moist gauze swab will detach most of the lesion. The laser can then be applied again to any residual islands of adherent epithelium.

illomaviral infections are then outlined, before the acetic acid reaction fades.

Collection of biopsy specimens. Provided that the correct technique is used, the carbon dioxide laser is an ideal tool for collecting additional biopsy specimens. Since the emissions from the carbon dioxide laser are not hemostatic for vessels larger than 1 mm, the stalks of any condylomas to be excised must first be compressed by means of a fine Vicryl or catgut ligature. The condyloma can then be excised with scissors, and the arterioles in the base of the condylomas sealed by lasing to the pedicle.

Since the edges of any laser crater tend to shrink toward the eenter of the impact site, the laser is of value as a thermal scalpel only if it is used with skillful traction and countertraction. Hence, sessile condylomas or acetowhite plaques are excised by first grasping the lesion with fine tissue forceps and having an assistant apply countertraction to the surrounding skin. The laser can

then be used to collect a bloodless biopsy specimen, by undercutting the plaque through a deep dermal or subcutaneous plane (Fig. 2). At the completion of the operation, these biopsy sites should be closed with fine Vicryl sutures.

Application of the laser to any foci of vulvar intraepithelial neoplasia. First, the laser is used to expose the proliferating zone of the epidermis by "brushing" the skin surface with a layer of laser energy, as previously described.⁷ The epidermal debris can now be removed from the operative field by gently wiping with a moistened gauze swab, thereby revealing smooth pink-white basement membrane overlying the anatomically intact papillary dermis. It is now an easy task to vaporize down to the third surgical plane (Fig. 1).

Lasing to the second plane is accomplished by moving the laser beam quickly enough to scorch, rather than crater, the exposed dermal surface. Such oscillations are done at about the same speed as used for "brushing" the epidermis. In contrast, lasing to the third plane is done by slow deliberate movements, controlling depth of penetration by careful hand-eye coordination.

Ablation of remaining condylomas and adjacent subclinical papillomaviral infection. The surgeon should not attempt to remove the remaining condylomas by undercutting them with the laser. Except for long-standing lesions (which can become quite pedunculated), most condylomas have a flat base. Attempts to undercut condylomas that are not required for histologic examination produce unnecessary dermal defects, and can be attended by troublesome bleeding (especially in pregnancy). Rather, the strategy should be to umbilicate the center of each lesion, debride the loosened epidermis, and relase to any residue (Fig. 3). Each condyloma should be umbilicated by lasing to the center and allowing tissue shrinkage at the laser impact site to pull the edge of the lesion into the operative field. It is unnecessary to lase to the edge of each condyloma; hence, the problem of unwanted damage to adjacent skin is easily avoided. The initial application of the laser should extend the vaporization crater to about the level of the surrounding skin, but there should be no penetration of the basement membrane at this point. Although it will be visually apparent that the zone of vaporization has not yet destroyed all of the abnormal keratinocytes at the base of the condyloma, lasing to this level will separate most of these cells from the basement membrane. Hence, this technique will minimize the extent of any unwanted thermal damage within the superficial dermis. Once the condylomas have been umbilicated, the area is easily debrided by gentle wiping with a moist gauze swab. Any residual epithelial fronds or capillary spikes can then be accurately destroyed by spot lasing.

Before debridement of the residues of these con-

dylomas, it is convenient to ablate any surrounding areas of subclinical infection to the first surgical plane (Fig. 1).

Using the laser within the vagina. If the cervical transformation zone shows papillomaviral infection or intraepithelial neoplasia, it is ablated to a measured depth of 7 mm, with the use of a power density of 750 to 1500 w/cm². To ensure destruction of the upper limit of the transformation zone, the treatment area is domed another 5 mm proximally, along the axis of the canal.¹³

Extensive papillomaviral infection may necessitate treatment of the vault, the lower third, or the entire vagina. To avoid any risk of bladder or rectal injury. the depth of destruction must be kept within the submucosa. Control of depth is achieved by lasing until the surface epithelium chars, with the use of a power density of 750 to 1200 w/cm².6 The vaginal side walls are exposed between the open blades of a bivalve speculum. Successive rotations of the speculum will expose the anterior and posterior walls. In the lower part of the vagina, a better angle of impact is obtained by aiming through the sides (rather than the central aperture) of the speculum. After the entire circumference has been treated, the speculum is withdrawn and the epithelial debris is wiped away by vigorous swabbing with a moist gauze square. When the speculum is reinserted, any untreated areas are easily identifiable, and can be ablated under direct vision. Recognition of residual islands of intact epithelium is sometimes aided by staining the vaginal walls with Lugol's iodine. Such islands stain mahogany brown and stand out against the unstained stroma.

Using the laser within the anal canal or urethra. Ablation within the anal canal or urethra requires special caution to limit the depth of destruction to the basement membrane. Circumferential destruction of the epithelium and submucosa will lead to anal or urethral stenosis. However, the technique of surface charring, debridement, and localized reapplication of the laser negates these risks. Low-power densities (350 to 450 w/cm²) are recommended. The anal canal can be visualized with a large nasal speculum, a small Peterson vaginal speculum, Sim's anoscope, or two pairs of uterine polyp forceps. Because of a theoretical risk of explosion, any flatus should be sucked from the rectum before the laser is used within the anus.

Exposure within the urethra is best done by using an endocervical speculum, or a small nasal speculum. If the surgeon is using a superpulsed laser, he should take advantage of this adaptation to minimize the zone of thermal coagulation within the urethra.¹⁴

Postoperative care. To counteract the inflammation and edema provoked by mild thermal injury to the underlying tissues, the vulva is liberally dressed with



Fig. 4. Photograph taken 10 days after laser vulvectomy, showing how healing should be advanced and almost complete. Application of the laser to an excessive depth is characterized by a relative delay in early healing, and an end result that *is* distinguishable from normal skin.

0.1% triamcinolone cream, while the patient is still in the lithotomy position.¹³

Following the empiric observation that urinary diversion will prevent a great deal of anticipated post-operative pain, patients who require large areas of vulvar ablation are offered a suprapubic catheter with leg bag for 2 weeks. Malodor is prevented by the daily instillation of 1 ounce of white vinegar into the collecting bag. Safeguards against bacterial infection include the prescription of prophylactic antibiotics and the daily cleansing of the collection system with household detergents. The catheter is removed at an office visit, when healing is well advanced.

Except in pregnant patients, treatments are performed as outpatient operations. Before discharge, each patient must have her ability to micturate normally verified by the recovery room nurse. Patients who void frequent small amounts should be specifically checked for retention with overflow. In the latter case it will be necessary to insert an indwelling Foley catheter. Before discharge, patients must also receive a prescription for a schedule 3 narcotic, a complete set of postoperative instructions, and an appointment for office follow-up in 1 week.

The most important part of the postoperative regimen is to soak for 30 minutes every 4 hours in a bath of reconstituted sea water (Instant Ocean)⁴ or hypertonic Epsom salt solution (1 cup per gallon). The vulva

Table II. A guide to the best level of destruction for different disease entities

Surgical plane	Appropriate for			
First	Condyloma acuminatum			
	Flat subclinical lesions			
Second	Micropapillary subclinical lesions			
	Hypertrophic dystrophies			
Third	Intraepithelial neoplasia, pilose-			
	baceous glands not involved			
Fourth	Intraepithelial neoplasia, involv- ing pilosebaceous glands			

should then be dried with a hair dryer, and dressed with a thin application of neomycin-bacitracin ointment. A prescription of Nupercainal 2% gel and a stool softener will make defecation less uncomfortable. Prophylactic antibiotics are not necessary, except to cover any indwelling Foley catheter.

Patients must be seen weekly for 3 weeks, to correct any early coaptation of adjacent raw surfaces. Healing should be virtually complete within 14 to 21 days (Fig. 4). Thereafter, women with refractory condylomas should return every 2 to 4 weeks for the next 3 months, so that any focal recurrences can be controlled by caustic agents. Because women with vulvar neoplasia or papillomaviral infections are at high risk for developing squamous neoplasia at other sites within the genital tract, surveillance by annual Papanicolaou smears is mandatory. 15, 16

Comment

Lasers are powerful but predictable surgical tools. Used with skill and discretion, they open new vistas in many medical specialties. However, despite their unique properties, careless or unskilled laser operations will yield undesirable results. Misadventure may arise (1) from accidental injuries to staff or patients, (2) by selection error, or (3) through the occurrence of conventional surgical complications.11 Avoidance of accidental injury requires an awareness of the path of the laser beam (from source to dissipation), and the exclusion of flammable drapes, plastic speculums, or dry swabs from the operative field. The major selection error is failure to detect occult invasion. Safeguards are a sound knowledge of lower genital tract neoplasia, colposcopic expertise, and strict adherence to triage rules. The risk of treatment failure is minimized by setting surgical margins with the colposcope, by individualization of depth according to histologic findings, and by treating focal recurrences of papillomaviral infection with topical trichloroacetic acid. Skillfully executed, this technique of superficial laser vulvectomy carries a very low rate of complications. However, less precise methods can lead to delayed healing, atrophic or hypertrophic scar formation, and vulvar coaptation.

Although safe and effective, superficial laser vulvectomy subjects patients to the risks of anesthesia and involves an absence from work or domestic duties of 10 to 14 days. 6.7 Hence, surgeons should satisfy themselves that the extent and severity of the problem are sufficient to warrant this operation. Since occasional malignant progression of carcinoma in situ has been reported in young, immunocompetent women,14 the eradication of vulvar intraepithelial neoplasia can be justified on the grounds of cancer prophylaxis, provided that nonmutilating methods are used.2.3 Hence, superficial laser vulvectomy is usually my treatment of choice for extensive or multifocal carcinomas in situ. However, the risk of malignant progression of hyperplastic dystrophies (with or without mild atypia) is too remote to justify operations on these same grounds. Rather, this diverse group of dermatologic disorders should be treated with topical steroids,2 with laser operations being reserved for those that continue to cause intractable itching or discomfort. Likewise, I believe that only refractory or extensive condylomas should be treated by laser ablation.6 However, when this decision has been made, the systematic approach outlined in this series of articles would appear to be more logical than the simple use of the laser as a "spot welder."

An important factor in poor outcome with the laser is the prevalence of a naive attitude that a favorable result is guaranteed by the technical sophistication of this tool. Unhappily, this is not true. Quality of outcome depends upon surgical precision (with the use of strategies that infer actual depth of thermal necrosis from the visual characteristics of more superficial landmarks), and accurate pathologic assessment (providing a rational basis for selecting different depths of destruction for different disease entities) (Table II). Once mastered, this method will consistently yield optimum results and allow confident preoperative counseling about the speed and cosmetic quality of eventual healing.

The technique of superficial laser vulvectomy is an exacting one that requires both careful attention to detail and considerable facility with the carbon dioxide laser. Inexperienced laser surgeons should not, under any circumstances, attempt this operation. As a generalization, laser operations performed by less experienced physicians tend to be too shallow on the cervix, but too deep on the vulva. Such habits can be difficult

to break. Hence, even experienced laser surgeons should ideally receive preceptorship in this procedure. If this is not possible, they should at least arrange to see a film that explicitly details the operative technique.

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The fetal biophysical profile in patients with premature rupture of the membranes—An early predictor of fetal infection

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A modified fetal biophysical profile (nonstress test, fetal movements, fetal breathing movements, fetal tone, amniotic fluid volume, and placental grading) was serially assessed in 73 patients who presented with premature rupture of the membranes and were not in labor. The last study before delivery was compared with the outcome of pregnancy. The relationships between individual variables and combinations of variables (biophysical scoring) and the outcome of pregnancy—as reflected by the development of chorioamnionitis and/or neonatal sepsis—were determined. These data suggest that the fetal biophysical profile is a useful tool for evaluating patients with rupture of the membranes. Rupture of the membranes by itself does not alter the biophysical scoring of the healthy fetus; however, a low biophysical score (≤7) was a good predictor of impending fetal infection in patients with premature rupture of the membranes. (AM J OBSTET GYNECOL 1985;152:510-6.)

Key words: Fetal biophysical profile, premature rupture of membranes, fetal infection

The fetal biophysical profile (nonstress test, fetal movements, fetal breathing movements, fetal tone, amniotic fluid volume, placental grading) has been shown to be more accurate in the identification of the hypoxic fetus than any other single test during the antepartum period. Its predictive value in antepartum fetal surveillance has been very well documented.^{1, 2} However, no data are available to support the use of the fetal biophysical profile in patients with premature rupture of the membranes.

The purpose of this prospective study was to determine the value of the fetal biophysical profile in evaluating patients who presented with premature rupture of the membranes and were not in labor. In this study, six fetal biophysical variables (nonstress test, fetal movements, fetal breathing movements, fetal tone, amniotic fluid volume, placental grading) were measured by a method previously described, to determine the relationships between single variables or combinations of variables and the outcome of pregnancy. Measures of pregnancy outcome included the presence of amnionitis and/or neonatal sepsis.

Material and methods

A total of 73 patients entered the study. The majority of these patients (94.5%) were transferred to the Uni-

versity of Connecticut Health Center because of preterm rupture of the membranes. Only singleton pregnancies with gestational ages ≥25 weeks, premature rupture of the membranes, and no labor were included. Patients with signs of labor, chorioamnionitis, bleeding, or fetal distress were excluded. The prior use of tocolysis and the presence of other complicating factors (for instance, pregnancy-induced hypertension, diabetes, intrauterine growth retardation) were also considered as contraindications for entering the study.

In all study patients, rupture of the membranes was documented by sterile speculum examination with pooled fluid, ferning, and alkaline pH determination (Nitrazine paper). Bimanual examination had been performed on several patients prior to transfer, but none was done after admission to our institution. Routine vital signs were obtained on admission. Electronic fetal heart rate monitoring was used to detect signs of fetal distress, as well as to document any uterine activity. If there was no evidence of labor, fetal distress, or infection, the fetal biophysical profile was determined with a technique previously described. The ultrasound evaluation was done by means of a linear array realtime ultrasound method (Picker LS-2000) equipped with a 3.5 MHz transducer. Real-time scanning consisted of a maximum 30-minute observation period during which fetal movements and fetal breathing movements were counted and fetal tone, amniotic fluid volume, and placental grading were estimated. Each biophysical variable (modified from the biophysical scoring of Manning et al.2) was scored as 2, 1, or 0 according to the criteria cited in our previous study.1 The maximal score was 12 and the minimal 0. The patients were advised that the results of the biophysical

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Table I. Predictive value of the biophysical scoring for development of infection in groups 1, 2, and 3

	ni 41 sisat	N		Infection	
Group	Biophysical score	No. of patients	No.	%	p value
Group 1 (53 patients)	≥8	37	1/37	2.7	
,	≤ 7	16	15/16	93.7	< 0.001
Group 2 (15 patients)	≥8	15	2/15	13.3	< 0.01
, ,	≤7	0	-	-	-
Group 3 (5 patients)	≥8	3	3/3	100	
The state of the s	≤7	2	1/2	50	Name:

scoring would not influence further management, since there are no data to support its usefulness in patients with premature rupture of the membranes.

The patients were observed in the hospital and were managed conservatively with bed rest and bathroom privileges. Corticosteroid therapy was administered in the majority of patients (60 of 73). Gestational ages were confirmed by complete ultrasound profiles. Temperature was taken four times a day and white blood cell counts were performed daily. Bimanual pelvic examinations were avoided unless the patient was believed to be in active labor or a decision had been made to induce labor because of amnionitis. The biophysical profile determinations were repeated every 24 to 48 hours if patients remained undelivered. A low biophysical score (≤7) was not an indication for delivery. Indications for delivery included labor, diagnosed amnionitis, persistent spontaneous variable decelerations (moderate to severe) on electronic fetal heart rate monitoring, or gestational age >37 weeks after a 24-hour period of observation if the cervix was favorable. The clinical diagnosis of amnionitis was made in the presence of two or more of the following criteria: Maternal fever >37.8° C, maternal tachycardia (120 bpm or more), leukocytosis (white blood cell count ≥20,000/mm³ in the absence of prior corticosteroid administration), fetal tachycardia (>160 bpm), uterine tenderness, and foul-smelling amniotic fluid. Antibiotics were administered to the mother only after clamping of the cord. One patient who was given antibiotics before cord clamping was excluded from the study.

On admission to the neonatal intensive care unit, all infants had cultures performed (blood, urine, cerebrospinal fluid, tracheal, nasogastric, external ear). All neonates at <34 weeks' gestation received prophylactic antibiotics (ampicillin and kanamycin), which were discontinued when cultures demonstrated no growth. Neonatal sepsis was diagnosed only in the presence of positive cultures of blood, urine, or cerebrospinal fluid. Possible neonatal sepsis was diagnosed in the absence of positive cultures when two or more of the following criteria were present: White blood cell count <5000/mm³, polymorphonuclear leukocyte count <1800/mm³, I:T ratio (ratio of bands to total neutrophil count) >0.2, or positive gastric aspirate for poly-

morphonuclear leukocytes showing >5 per high-power field. When neonatal sepsis or possible neonatal sepsis was diagnosed, the antibiotics were continued for approximately 7 to 10 days.

Data collected after delivery included Apgar scores, birth weight, cord blood pH (umbilical artery and umbilical vein), route of delivery, placental cultures, and neonatal septic workup. For the purpose of this report, infection outcome was defined as the presence of clinical amnionitis, possible neonatal sepsis, or neonatal sepsis. The last study before delivery was compared with the outcome of pregnancy, as reflected by the development of infection (amnionitis, possible neonatal sepsis, neonatal sepsis). The association of each biophysical variable as well as combinations of variables (biophysical scoring) and the outcome was determined.

Results

There were 148 examinations in these 73 patients (2.02 examinations per patient). The earliest gestational age tested was 25 weeks, the latest was 41 weeks, and the mean was 31.9 weeks. Nine examinations were the most done on one patient. The average time of the ultrasound fetal biophysical assessment was 15 minutes.

Of the 73 pregnancies, 69 (94.5%) were preterm (<37 completed weeks) and four (5.4%) were term (>37 weeks) at delivery. In only two patients was labor induced for the presence of a favorable cervix after 37 weeks' gestation and failure to go into spontaneous labor after a 24-hour period of observation. The mean prolongation of pregnancy was 6.7 days and the infection rate (amnionitis, possible neonatal sepsis, neonatal sepsis) was 30.1% (22 of 73 patients).

Of the 73 patients, 53 (72.6%) were delivered within 24 hours of the final examination (group 1), 15 (20.5%) between 24 and 48 hours (group 2), and five (6.8%) >48 hours from the last examination (group 3). The predictive value of the biophysical scoring for the development of infection is illustrated in Table I (χ^2 analysis). As can be seen, group 3 was too small to derive any meaningful statistical conclusions; however, it appears that there was no predictive value of the biophysical score in patients who were delivered >48 hours after the last examination. In group 1, a biophysical score of ≥ 8 was associated with an infection

BIOPHYSICAL SCORE															TOTAL NO. OF PATIENTS = 53
12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	(14)
11	0	0	0	0	0	0									(6)
10	+	0	0	0	0										(5)
9	0	0	0	0	0	0									(6)
8	0	0	0	0	0	0									(6)
7	•	X	0												(3)
6		•	•	Х	Х	+									(6)
5															(1)
4	•	•													(2)
3	•	•													(2)
2		A													(2)

Fig. 1. Scatter diagram of the distribution of individual scores of both infected and noninfected cases of group 1. Number of patients is shown in parentheses. ○ = Noninfected. ● = Amnionitis and neonatal sepsis. ■ = Neonatal sepsis. ▲ = Amnionitis and possible neonatal sepsis. X = Possible neonatal sepsis. + = Amnionitis.

Table II. Mean ± 2 SD of the biophysical score, Apgar scores <7, umbilical cord pH, and gestational age of the infected versus the noninfected cases of group 1

			Apgar se	cores <7		Umbilica	l cord pH	
No. of	Biophysical	1 n	iin	5 1	nin			0.15.1
patients score	• -	No.	%	No	%	Artery	Vein	Gestational age (wk)
Infected (16) Noninfected (37) p value	5.2 ± 4.0 10.3 ± 3.2 < 0.01	10/16 8/37 <0.	$62.5 \\ 21.6 \\ 01$	8.16 1.37 <0	50 2.7 .01	7.29 ± 0.14 7.31 ± 0.10 NS	7.34 ± 0.12 7.36 ± 0.10 NS	30.2 ± 6.0 32.7 ± 6.1 <0.01

Total number of patients = 53.

rate of 2.7%, and a low biophysical score (\leq 7) was associated with an infection rate of 93.7% (p < 0.001). In group 2, a biophysical score of \geq 8 was associated with an infection rate of 13.3%. This was significantly lower than the overall infection rate of 30.1% (p < 0.01). Subsequently, groups 2 and 3 were eliminated and further statistical analysis was therefore confined only to group 1.

Of the 16 infected cases in group 1, delivery was performed in three because of persistent spontaneous variable decelerations, in five because of diagnosed antepartum amnionitis, in five because of simultaneous onset of labor and amnionitis, and in three because of spontaneous onset of labor alone. Of the 37 noninfected cases of group 1, delivery was performed in 28 patients because they went into labor, in seven because of persistent spontaneous variable decelerations, and in two for the presence of a favorable cervix at a gestational age of ≥37 weeks and no labor after a 24-hour period of observation. The distribution of individual biophysical scores is provided in Fig. 1 (scatter diagram) for both infected and noninfected cases of group 1. Table II illustrates the mean ± 2 SD of the biophysical score, Apgar scores, umbilical cord pHs, and gestational ages of the infected versus the noninfected cases of group 1 (Wilcoxon signed rank test). The infected group had significantly lower mean biophysical scores and Apgar scores (1 and 5 minutes) and earlier ges-

tational ages (<0.01). There was no significant difference in cord pH between the infected and noninfected cases. The final study before delivery was compared with the outcome of pregnancy as reflected by the development of amnionitis, possible neonatal sepsis, or neonatal sepsis (infection). The relationship between each biophysical variable and infection outcome is illustrated in Table III. A stepwise logistic regression analysis was used to determine the value of the biophysical score and the value of each biophysical variable in order of importance in predicting infection versus noninfection outcome. The best predictor of infection was found to be the biophysical score (F statistic 96.22). Of the individual biophysical variables, the nonstress test, fetal breathing movements, fetal movements, and fetal tone were found to be important in the above sequence (F statistic values 20.97, 20.38, 13.57, 12.18). Placental grading was not found to have any significant predictive value. The relationship between the combination of variables (mean biophysical scoring) and the different infection outcomes is shown in Table IV. As can be seen, the lowest biophysical scores were encountered in patients with positive neonatal cultures (neonatal sepsis). The biophysical profile of the infected cases is analyzed in Table V. As can be seen, when fetal movements were compromised (fetal movement score 1 or 0), the recovery of positive neonatal cultures (neonatal sepsis) was 75% (six of eight). There were a total

Table III. Relationship between each biophysical variable and infection outcome (group 1)

en trade de la companya de la compa		m , 11. 4	Infe	cted	Nonin	fected
Biophysical variable	No. of patients*	Total last tests (%)	No.,†	%‡	No.§	%
NST 2	27	50.9	1/27	3.7	26/27	96.2
NST 1	8	15.0	2/\$	25.0	6/8	75.0
NST 0	18	33.9	13/18	72.2	5/18	27.7
FBM 2	24	45.2	0/24	0.0	24/24	100.0
FBM 1	3	5.6	0/3	0.0	3/3	100.0
FBM 0	26	49.0	16/26	61.5	10/26	38.4
FM 2	43	81.1	8/43	18.6	35/43	81.3
FM 1	4	7.5	2/4	50.0	2/4	50.0
FM 0	6	11.3	6/6	100.0	0/6	0.0
FT 2	46	86.7	9/46	19.5	37/46	80.4
FT 1	5	9.4	5/5	100.0	0/5	0.0
FT-0	2	3.7	2/2	100.0	0/2	0.0
AF 2	27	50.9	3/27	11.1	24/27	88.8
AF 1	13	24.5	4/13	30.7	9/13	69.2
AF 0	13	24.5	9/13	69.2	4/13	30.7
PL 2	50	94.3	14/50	28.0	36/50	72.0
PL I	i	1.8	1/1	100.0	0/1	0.0
PL 0	2	3.7	1/2	50.0	1/2	50.0

NST = Nonstress test; FBM = fetal breathing movements; FM = fetal movements; FT = fetal tone; AF = amniotic fluid; PL = placental grading.

Percent of noninfected cases = 69.8.

of seven instances of positive neonatal cultures. Of these, four were recovered from the blood (two group B Streptococcus, one Bacillus sp., and one Escherichia coli), two from the urine (one positive for group B Streptococcus and one positive for Peptostreptococcus), and one from cerebrospinal fluid (group B Streptococcus).

There were only two neonatal deaths in the population tested. Both infants were delivered at 26 weeks' gestation because of amnionitis and severe variable decelerations. The last biophysical scores before delivery were 2 and 3, respectively (perinatal mortality 27.3/ 1000).

Comment

Premature rupture of the membranes constitutes one of the most common complications of pregnancy. In the literature, the reported incidence ranges from 5% to 40%.3.4 Although premature rupture of membranes before 37 weeks' gestation is hazardous to the fetus in terms of perinatal morbidity and mortality, its occurrence at term (after 37 weeks) also subjects the mother and fetus to increased risks.5

Many investigators recommend prompt delivery of the fetus when premature rupture of the membranes occurs at term, because of the increased incidence of amnionitis with prolonged rupture of the membranes.6 However, this action will usually result in an increased incidence of primary cesarean sections for failed induction of labor and cephalopelvic disproportion.5

Table IV. Relationship between mean biophysical score and different infection outcomes (group 1)

Diagnosis	No. of patients	Mean biophysical score
Amnionitis	2	8.0
Possible neonatal sepsis	3	6.3
Amnionitis-possible neonatal sepsis	4	5.5
Neonatal sepsis	3	4.3
Amnionitis-neonatal sepsis	4	3.5

As the biophysical score decreased, the severity of the infection increased (Spearman rank correlation -0.75, $p \le 0.001$).

When premature rupture of the membranes occurs in a preterm gestation (<37 weeks), the management is even more controversial. In preterm gestations, immediate delivery of the fetus carries a significant risk of hyaline membrane disease, which still remains a maor contributor to neonatal morbidity and mortality; therefore conservative management has been advocated by previous investigators.7 However, the conservative approach carries an increased risk of fetal/neonatal infection and cord prolapse. Other management protocols involve the use of amniocentesis8,9 in patients with preterm rupture of the membranes to detect not only the fetus with a mature lung profile but also the fetus most likely to develop sepsis. The problems as-

^{*}Total number of patients = 53.

[†]Total number = 16.

[‡]Percent of infected cases = 30.1.

[§]Total number = 37.

Table V. Analysis of biophysical profile of 16 infected cases (group 1)

Case No.	NST	FBM	FM	FT	AF	PL	Total score	Diagnosis
1	2	0	2	2	2	2	10	Amnionitis
2	. 0	0	2	1	i	2	6	Amnionitis
3	- 0	0	2	2	1	2	7	Possible neonatal sepsis
4	0	0	2	2	0	2	6	Possible neonatal sepsis
5	0	0	2	2	0	2	6	Possible neonatal sepsis
6	0	0	2	2	1	2	7	Amnionitis-possible neonatal sepsis
7	1	0	0	1	2	2	6	Amnionitis-possible neonatal sepsis
- 8	0	0	2	2	0	2	6	Amnionitis-possible neonatal sepsis
9	0	0	0	1	0	1	2	Amnionitis-possible neonatal sepsis
10	0	0	1	2	1	2	6	Neonatal sepsis
11	1	0	2	0	2	0	5	Neonatal sepsis
12	0	0	0	0	0	2	2	Neonatal sepsis
13	0	θ	0	2	0	2	4	Amnionitis-neonatal sepsis
14	.0	0	1	1	0	2	4	Amnionitis-neonatal sepsis
15	0	0	0	2	0	1	3	Amnionitis-neonatal sepsis
16	0	0	0	1	0	2	3	Amnionitis-neonatal sepsis

NST = Nonstress test; FBM = fetal breathing movements; FM = fetal movements; FT = fetal tone; AF = amniotic fluid; PL = placental grading.

sociated with this approach have been the inability to obtain fluid in almost half of these patients,8 the invasiveness of the procedure, and the lack of strong correlation between the presence of bacteria in the amniotic fluid and fetal sepsis.8.9 The presence of bacteria in the amniotic fluid (Gram stain and culture) obtained by transabdominal amniocentesis has been strongly correlated with the development of amnionitis and/or postpartum endometritis but not with fetal/neonatal sepsis as determined by the presence of positive neonatal cultures (blood, cerebrospinal fluid, urine). The incidence of fetal/neonatal sepsis with positive amniocentesis was only 14.2% (one of seven) in the study of Cotton et al.9 and 22.2% (two of nine) in that of Garite et al.8 In summary, a uniform approach to the management of patients with premature rupture of the membranes has not yet been elucidated.

Until recently, the antepartum fetal evaluation of patients with premature rupture of the membranes has been solely confined to nonstress testing. The contraction stress test is contraindicated and has no place in the antepartum fetal evaluation of these patients because of the theoretical risk of initiating labor. The value of the fetal biophysical profile has been documented1 in high-risk patients with intact membranes; however, its value in the antepartum fetal evaluation of patients with premature rupture of the membranes has not been previously determined. The relatively high neonatal sepsis rate in our study population is probably the result of the expectant management of premature rupture of the membranes in a selected group of high-risk patients referred to a tertiary care center. The inclusion of the possible neonatal sepsis group in the present study is in accordance with the current pediatric and obstetric literature. 10-12 This

group is usually defined as the group with strong clinical and laboratory evidence of bacterial infection but with negative cultures (blood, cerebrospinal fluid). The negative cultures in this group of infants may be related to inadequate culturing techniques or the inherent difficulty encountered by most laboratories in isolating anaerobic bacteria. Nevertheless, these infants are usually treated after birth with intensive antibiotic therapy as are infants with positive cultures. The inclusion of the "possible neonatal sepsis" group in our study therefore allows comparison with other studies because it was clearly defined according to published data. 10-12

According to our data, the rupture of membranes by itself does not alter the biophysical activities of the healthy fetus. However, a low biophysical score (≤7) was a good predictor of impending fetal infection in patients with premature rupture of the membranes. In cases of impending fetal infection, the fetal biophysical activities are altered in a manner very similar to alteration of activities in the hypoxia of uteroplacental insufficiency. The first manifestations of impending fetal infection were nonreactive nonstress testing and absence of fetal breathing. Loss of fetal motion and poor fetal tone were late signs of fetal infection, since the majority of these infants had positive cultures in the immediate neonatal period (Table V). The presence of fetal breathing (Table III) had the highest specificity in predicting absence of fetal infection. There were no cases of fetal infection when breathing was present within 24 hours prior to delivery. It has been reported that the incidence of fetal breathing movements is decreased prior to the onset of labor, but these observations were made in chronic fetal lamb preparations.13 It is not yet clear whether fetal breathing movements are diminished prior to labor in humans. The data of

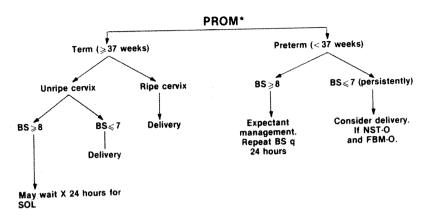


Fig. 2. Proposed protocol for management of premature rupture of the membranes. *Premature rupture of the membranes. BS = Biophysical score. NST-0 = Nonreactive nonstress test. FBM- θ = Fetal breathing absent. SOL = Spontaneous onset of labor.

Patrick and Challis,14 however, which are based on human fetal breathing movements, suggest that, prior to normal spontaneous labor in humans, fetal breathing movements may be decreased but that when labor follows spontaneous rupture of the membranes—as in our study population—fetal breathing movements do not decrease until accelerated labor occurs. In fact, these investigators reported an increase in fetal breathing movements in two fetuses which were observed during early labor. In our study group (group 1) there was a lower incidence of spontaneous onset of labor in the infected group (eight of 16, or 50%) versus the noninfected group (28 of 37, or 75.6%). Therefore the absence of fetal breathing in the infected group cannot be attributed to impending spontaneous onset of labor but rather to fetal infection. The relationship between gestational age and fetal biophysical profile scoring has been investigated in our institution by a recent retrospective study of 1151 biophysical profiles and scores of pregnancies with good outcome. In that study [Vintzileos AM, Feinstein SJ, Lodeiro JG, Campbell WA, Weinbaum PJ, Nochimson DJ. The biophysical profile of the healthy fetus from 25 to 44 weeks of gestation and the effect of premature rupture of the membranes (submitted for publication)] the incidence of fetal biophysical scoring of ≥8, as well as mean biophysical scoring, was found to remain unchanged from 25 to 44 weeks' gestation regardless of the status of the membranes. Therefore the low scores of the infected cases in the present study cannot be attributed to lower mean gestational age (Table II) but rather to fetal infection. A tendency for an increased incidence of infection with diminishing amniotic fluid volume was also observed (Table III). It could be speculated that patients with decreased amniotic fluid volume are deprived of the bacteriostatic effect of a normal amniotic fluid volume. The loss of fetal movement (fetal movement score 0) and fetal tone (fetal tone score 0) was the best predictor of fetal infection, but as it has been already stated, this is a late sign of infection. The decrease in fetal biophysical activities prior to the development of clinical infection makes sense, especially in cases of an ascending infection where the fetus seems to be the first target.

Fetal infection that may lead to acidosis and perinatal hypoxic-ischemic encephalopathy has already been described.15 The mechanism by which fetal infection diminishes fetal biophysical activities without acidosis, as determined by cord blood pH measurements, is unknown. Our speculation is that early fetal infection may increase fetal oxygen demands and cause local tissue hypoxia and, thereby, malfunction of the central nervous system centers that control the reflex biophysical activities.1 Only late stages of fetal infection are manifested by blood acidosis and possible neurological damage due to hypoxic-ischemic encephalopathy.15

When premature rupture of the membranes occurs at term, the fetal biophysical profile should be used to select those patients who are candidates for fetal infection and therefore in need of prompt delivery. In preterm rupture of the membranes, the use of the fetal biophysical profile could replace amniocentesis in selecting those patients who are less likely to develop fetal infection and may be benefited by expectant management. For the majority of these patients, labor may be arrested for at least 48 hours, the minimum period required to enhance fetal lung maturity with corticosteroids. Fig. 2 illustrates a proposed protocol which we currently use in an ongoing prospective study to test its efficacy in improving pregnancy outcome in patients with premature rupture of the membranes. A persistently low biophysical score (≤7) with a nonreactive nonstress test and absence of fetal breathing movements is highly predictive of fetal infection in patients with premature rupture of the membranes and has been used by us as an indication for immediate delivery. In summary, the fetal biophysical profile is a simple, noninvasive, and reliable tool for evaluating patients with premature rupture of the membranes.

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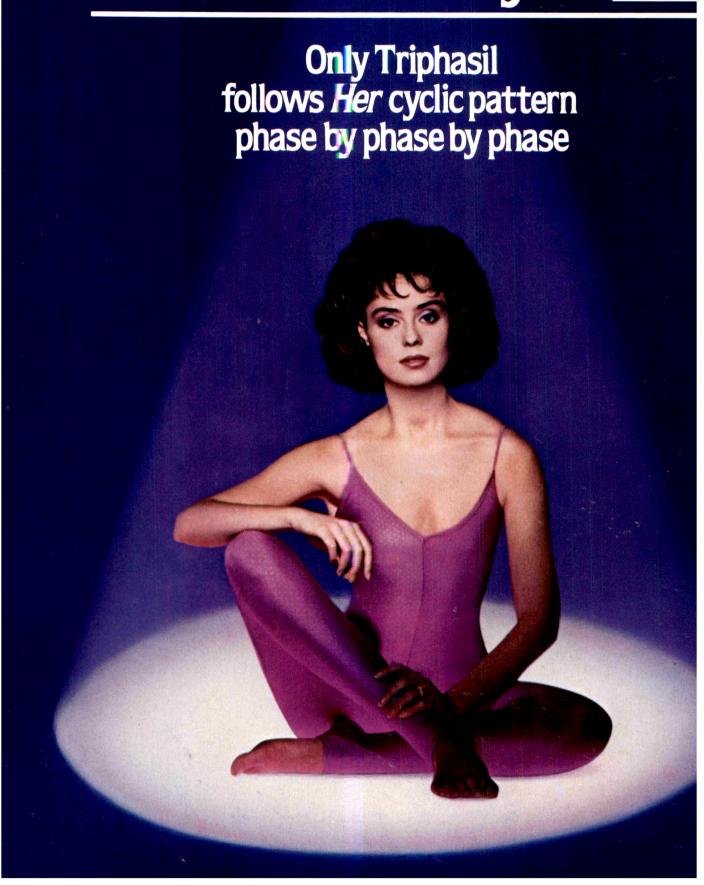
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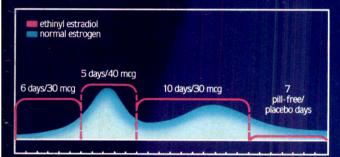
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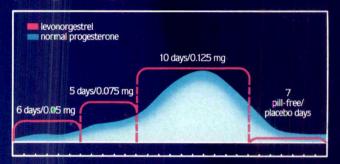


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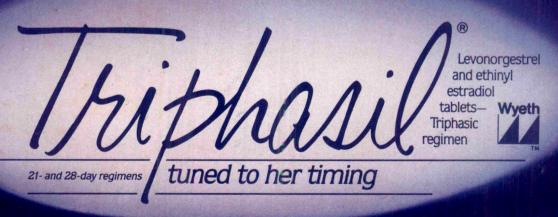
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No significant impact on lipid levels shown in 6- and 18-month studies

Two separate 6-month studies demonstrate minimal impact on lipid levels; no statistically significant changes were observed in HDL, LDL, total cholesterol or triglycerides. ^{1,2} This is further supported by a separate 18-month prospective study in which 85 non-smoking women under the age of 35 were equally divided among 4 oral contraceptive formulations. With Triphasil, no statistically significant changes in total or HDL cholesterol occurred. ³ In this study, slight increases in triglycerides, which peaked at cycle 9, declined in subsequent cycles.

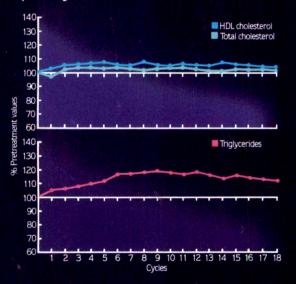
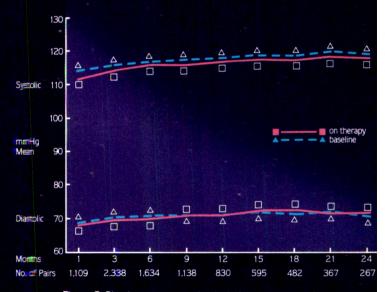


Figure 1: Monthly HDL cholesterol, triglycerides and total cholesterol levels for Triphasil over an 18-month period.

(Adapted from Briggs 3)

No significant impact on blood pressure means shown in 24-month study

In clinical trials over 2 years, Triphasil had no significant impact on blood pressure means. As shown in Figure 2, a high degree of stability was maintained with Triphasil compared to baseline.²



 $\label{eq:Figure 2: Blood pressure mean levels during treatment and baseline -- systolic and diastolic.$

No significant impact on carbohydrate metabolism in 18-month study

Results at 6, 12, and 18 months demonstrate Triphasil has little influence on carbohydrate metabolism. Glucose tolerance and insulin levels generally remain within normal clinical limits. Figure 3 shows glucose tolerance test results from a study of 85 non-smoking women under the age of 35 who were equally divided among 4 oral contraceptive formulations. Blood samples were taken before Triphasil and at treatment cycles 6, 12, and 18. Findings at 18 months were not plotted because they were comparable to 12-month data.3

hours 100 hours

Figure 3: Oral glucose tolerance test results: Pretreatment and during treatment cycles 6 and 12.

And a low level of minor side effects-including breakthrough bleeding*

The more natural phasing of both estrogen and progestogen in Triphasil provides oral contraception that is characterized by a low incidence of breakthrough bleeding and minimal complaints of other minor side effects. This is demonstrated in extensive multicenter clinical trials spanning more than three years.2

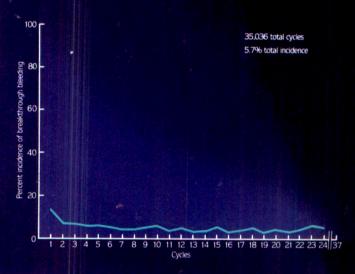
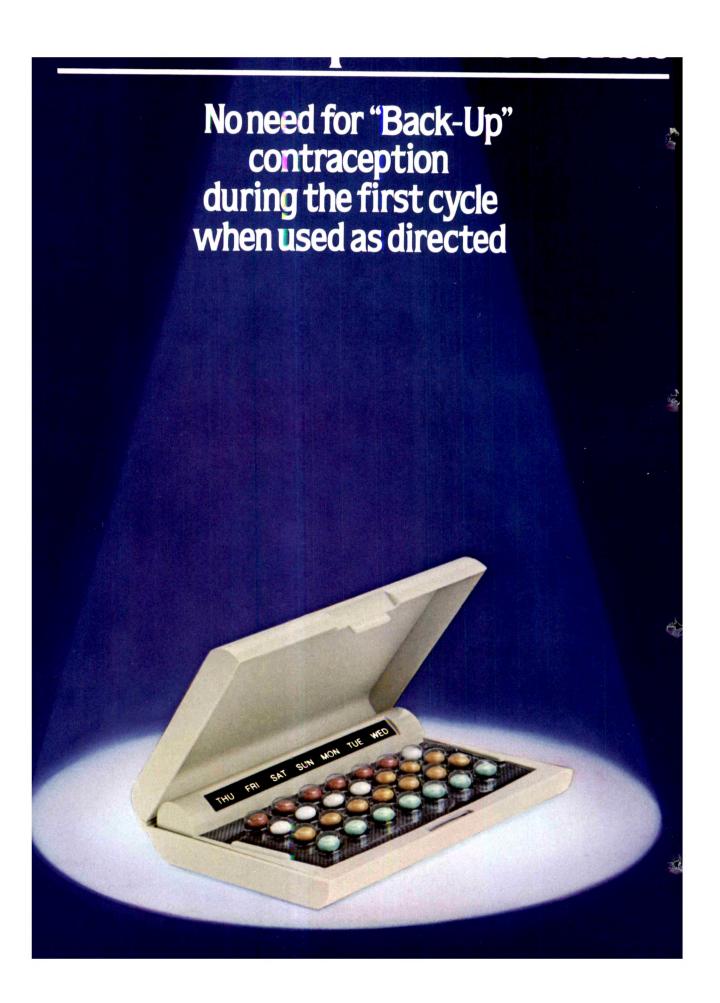


Figure 4: Incidence of breakthrough bleeding in 3,546 women during 24 months of Triphasil therapy.



21- and 28-day regimens

Worldwide experience confirms clinical studies



starts from dose 1, Herday 1

Unique Dosage Regimen: Starts on Her Day 1

Triphasil® gives oral contraception a simple new start—dose 1 begins on her day 1. For the first cycle she'll take the first tablet on her first day of bleeding—eliminating the confusion of counting days or waiting for Sunday. It's easy for her to remember... and for you to explain.

No Need for "Back-Up" Contraception During the First Cycle When Used as Directed

Unlike oral contraceptives which utilize a Sunday-start regimen, with Triphasil no additional protection was used during clinical studies in 3,546 women, and no pregnancies occurred during the first cycle. In worldwide post-marketing experience, there has been only one first cycle pregnancy reported in the literature. For dosage and administration, please refer to brief summary.

Day 1 Start— In Tune with *Her* Timing

Day 1 start means she starts on a day established by HER OWN biology, not by a calendar or package.

Elegant Compact—Discreet, Functional and Reusable

To keep it simple, Triphasil has a new package—a discreet, easy-to-follow reusable compact she'll feel comfortable with from day 1.



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IN BRIEF:
TRIPHASIL*—6 brown tablets containing 0.050 mg levonorgestrel with 0.030 mg ethinyl estradiol; 5 white tablets containing 0.075 mg levonorgestrel with 0.040 mg ethinyl estradiol; 10 light-yellow tablets containing 0.125 mg levonorgestrel with 0.030 mg ethinyl estradiol (7 light-green tablets containing inert ingredients are included in the 28-day regimen)—Triphasic regimen.

Indications and Usage—TRIPHASIL* is indicated for the prevention of pregnancy in women who elect to use oral contraceptives (OC's) as a method of contraception.

contraceptives (0C's) as a method of contraception.

Contraindications—OC's should not be used in women with any of the following conditions: 1. Thrombomhlebitis or thromboembolic disorders. 2. A past history of deep-vein thromboembolic of thromboembolic disorders. 3. Cerebral-vascular or coronary-artery disease. 4. Known or suspected carcinoma of the breast. 5. Known or suspected early or coronary-artery disease. 4. Known or suspected early or coronary-artery disease. 4. Known or suspected early or coronary-artery disease. 7. Known or suspected early or coronary-artery disease. 4. Known or suspected early or susp containing products

Warnings

Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptiwe use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not

The use of oral contraceptives is associated with increased risk of several serious conditions, including thromboembolism, stroke, myocardial infarction, hepatic adenoma, gallbladder disease, hypertension, Practitioners prescribing oral contraceptives should be familiar with the following information relating to these pisks.

1. Thromboembolic Disorders and Other Vascular Problems—An increased risk of thromboembolic and thrombotic disease associated with use of OC's is well established. Three principal studies in Great Britain and 3 in the U.S. have demonstrated increased risk of fatal and nonfatal venous thromboembolism and stroke, both hemorrhagic and thrombotic. These studies estimate that users of OC's are 4 to 11 times more likely than nonusers toudevelop these diseases without evident cause.

diseases without evident cause.

CFREBROVASCULAR DISORDERS—In a collaborative American study of cerebrovascular disorders in wimmen with and without predisposing causes, it was estimated that the risk of hemorrhagic stroke was 2.0 times greater in users than nonusers and the risk of thrombotic stroke was 4.0 o 5 times greater in users than in nonusers.

MYOCARDIAL INFARCTION (MI)—An increased risk of MI associated with the use of 0.0°s has been reported, confirming a previously suspected association. These studies, conducted in the UK, found, as expected, that the greater the number of underlying risk factors for coronary-artery disease (cigarette smoking, hypertensien, hypercholesterolemia, obesity, diabetes, history of pre-eclamptic toxemia) the higher the risk of development. Treatment of relative risk, it has been estimated that OC users who do not smoke (smoking) is considered a major predisposing condition to MI) are about twice as likely to have a fatal MI as nonusers who do not smoke. Ocusers-who are also smokers have about a 5-fold increased risk of fatal MI compared to users who do not smoke. Ocusers-who are also smokers have about a 5-fold increased risk of fatal MI compared to users who do not smoke but so the bout a 10-to 12-fold increased risk compared to nonusers who do not smoke but are also smokers have about a 5-fold increased risk compared to nonusers who do not smoke but are subsuit a 10-to 12-fold increased risk compared to nonusers who do not smoke but are subsuit a 10-to 12-fold increased risk compared to nonusers who do not smoke but are subsuit a 10-to 12-fold increased risk compared to nonusers who do not smoke but a remaining include the piece of the predisposing conditions mentioned above in determining importance of these relative risks, however, baseline rates for various age groups must be given serious consideration. Importance of other predisposing conditions mentioned above in determining relative and absolute risks has not as yet been quantified, quite likely the same synergis

given serious consideration. Importance of other predisposing conditions mentioned above in determining relative and absolute risks has not as yet been quantified; quite likely the same synerijstic action exists, but perhaps to a lesser extent.

RISK OF DOSE—In an analysis of data derived from several national adverse-reaction reporting systems. British investigators concluded that risk of thromboembolism, including coronary thrombosis, is directly retated to dose of estrogen in OC's. Preparations containing 100 mcg or more of estrogen were associated with higher risk of thromboembolism than those containing 50-80 mcg. Their analysis did suggest, however, that quantity of estrogen may not be the sole factor involved. This finding has been confirmed in the U.S.
ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASES—A large prospective study carried out in the UK estimated the mortality rate per 100,000 women per year from diseases of the circulatory system for users and nonusers of 00's according to age, smoking habits, and duration of use. Overall excess death rate annually from circulatory diseases for OC users was estimated to be 20 per 100,000 (ages 15-34–5100,000; ages, 35-44–33') 100,000; ages 45-49–140/100,000), risk being concentrated in older women, in those with long duration of use and in cigarette smokers. It was not possible, however, to examine interrelationships of age, smoking, and duration of use, nor to compare effects of continuous vs. intermittent use. Although the study showed a 10-floid increase in death due to circulatory diseases in users for 5 or more years, all these deaths occurred in women 55 or older. Until larger numbers of women under 35 with continuous use for 5 or more years are available, it is not pessible to assess magnitude of relative risk for this younger group. Available data from a variety of sources have been analyzed to estimate risk of death associated with various methods of contraception. Estimates of risk official increased risk of death associated with circulate vi

vision; onset of proptosis or diplopia; papilledema; or retinal-vascular lesions, and institute appropriate diagnostic and therapeutic measures.

3. Carcinoma—Long-term continuous administration of either natural or synthetic estrogen in certain animal species increases frequency of carcinoma of the breast, cervix, vagina, and liver. Certain synthetic progestogens, one currently contained in OCs. In which we have species increases frequency of carcinoma and malignant, in dogs. In humans, 3 case-control studies have reported an increased risk of endometrial carcinoma associated with prolonged use of exogenous estrogen in postmenopausal women. One publication reported on the first 21 cases submitted by physicians to a registry of cases of adenocarcinoma of the endometrium in women under 40.0n OCs. Of cases found in women without predisposing risk factors (e.g., irregular beleding at the time OCs were irrst given, polycystic ovaries), nearly all occurred in women without predisposing increased risk of endometrial cancer in users of conventional combination or progestogen-only OCs. Several studies have found no increase in breast cancer in women atking OCs or estrogens. One study, however, while also noting no overall increased risk of breast cancer in women on OCs, found an excess risk in subgroups of OC users with documented bening breast diseases. Reduced occurrence of bening breast tumors in users of OCs has been well documented. In summary, there is at present no confirmed evidence from human studies of increased risk of cancer associated with OCs. Close clinical surveillance of all women on OCs. is, nevertheless, essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be taken to rule out malignancy. Women with a strong family history of breastrancer or with breast nodules, fibrocystic disease, or abnormal mammograms should be monitored with particular are if they elect to use OCs.

with breast nodules, fibrocystic disease, or abnormal mammograms should be monitored with particular eare if they elect to use OC's.

4. Hepatic Tumors—Benign hepatic adenomas have been found to be associated with use of OC's. One-study showed that OC's with high hormonal potency were associated with higher risk than lower potency OC's. Although benign, hepatic adenomas may rupture and may cause death through intra-abdominal hemorrhage. This has been reported in short-term as well as long-term users. Two studies relate risk with duration of use of OC's, the risk being much greater later 4 or more years use. While hepatic adenoma is rare, it should be considered in women presenting abdominal pain and tenderness, abdominal mass or shock. A tew cases of hepatocellular carcinoma have been reported in women on OC's. Relationship of these drugs to this type of malignancy is not known.

5. Use in or Immediately Preceding Pregnancy, Birth Defects in Offspring, and Malignancy in Female leftspring—Use of female sex hormones—both estrogenic and progresational agents—during early pregnancy seenously damage the offspring. It has been shown that females exposed in utero to diethylstibestrol, a nonsteroidalestrogen, have increased risk of developing in later life a form of vaginal or cervical cancer ordinarily extremely rise. It is risk has been estimated to be of the order of 1 in 1,000 exposures or less. Although there is no evidence we that OC's further enhance risk of developing in later if a form of vaginal more into the way enterties of the vagina and cervix. Although these changes are histologically benign, it is not known whether this coolition is a precursor of vaginal malignancy. Such highers hosposed may develop abnormalities of the urgenital tract. Although similar data are not available with use of other estrogens, it cannot be presumed they would not induce similar changes. An increased risk of congenital anomalies, including heart defects and limb defects in sidnate enterties in infants exposed in utero to sex

tests for pregnancy, or attempted treatment for threatened abortion). Some exposures involved only a few days. Data suggest that risk of limb-reduction defects in exposed fetuses is somewhat less than. I in 1,000 live births the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and their is no evide from well-controlled studies that progestogens are effective. There is some evidence that trajloidy and possibly types of polyploidy are increased among abortuses from women who become pregnant son or atter casing OC's Embryos with these anomalies are virtually always aborted spontaneously. Whether there is an overall increase is reportaneously with these anomalies are virtually always aborted spontaneously. Whether there is an overall increase is reportaneously abortion of pregnancial some and the spontaneously with the pregnancy is not abortion of the prescribed schedule, the possibility of pregnancy should be considered at time of it missed period, and further use of OC's should be withheld until pregnancy has been ruled out. If pregnancy is confirmed, the patient should be apprised of the potential risks to the fetus, and advisability of continuation of the pregnancy should be discussed. It is also recommended that women who discontinue OC's with the pregnancy is should be discussed. It is also recommended that women who discontinue OC's wither of the pregnancy should be discussed. It is also recommended that women who discontinue OC's wither of the pregnancy should be discussed. It is also recommended that women who discontinue OC's with mist of the pregnancy should be discussed. It is also recommended that women who discontinue OC's with the pregnancy is a should be discussed. It is also recommended that the pregnancy should be discussed. It is also recommended that the pregnancy should be discussed. It is also recommended that the pregnancy should be discussed th

Precautions—GENERAL—1. A complete medical and family history should be taken prior to initiation of OC's. Pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Pap smear and relevant laboratory tests. As a general rule OC's should not prescribed for longer than 1 year without another physical examination and Pap smear.

2. Under influence of estrogen-progestogen preparations, preexisting uterine leiomyomata may increase in size.

3. Patients with history of psychic depression should be carefully observed and the drug discontinued if depressic recurs to a serious degree. Patients becoming significantly depressed while on OC's should stop OC's and use an alternate method to try to determine whether the symptom is drug-felated.

4. OC's may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention, such as convulsive disorder imgraine syndrome, asthma, or cardiac or renal insufficiency.

5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence while on OC's. If jaundice develops, OC's snould be discontinued.

6. Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administer.

Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered the caution

with caution.

7. OC users may have disturbances in normal tryptophan metabolism which may result in a relative pyridoxine deficiency. Clinical significance is undetermined.

8. Serum foliate levels may be depressed by OC's. Since the pregnant woman is predisposed to development of tolate deficiency and incidence of foliate deficiency increases with increasing gestation, it is possible that if a woma becomes pregnant shortly after stopping OC's, she may have a greater chance of developing foliate deficiency and complications attributed to this deficiency.

9. The pathologist should be advised of OC therapy when relevant specimens are summitted.

10. Certain endocrine- and liver-function tests and blood components may be affected by estrogen-containing OC

a. Increased sulfobromophthalein retention.
 b. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability.

c. Increased thyroid-binding globulin (TBG) leading to increased circulating total-thyroid hormone, as measured b protein-bound iodine (PBI). 14 by column, or 14 by radioimmunoassay. Free 13 resin uptake is decreased, reflect the elevated TBG: free 14 concentration is unaltered.

d. Decreased pregnanediol excretion.

u. Decleased pregnaneon excretion.

Reduced response to metyrapone test.

Information for the Patient—See Patient Package Labeling.

Drug Interactions—Reduced efficacy and increased incidence of breakthrough bleeding have been associated with concomitant use of Iritampin. A similar association has been suggested with barbiturates, phenylbutazone, phenylication, ampicillin and tetracycline.

Carcinogenesis—See Warnings section for information on carcinogenesis.

Pregnancy—Category X. See Contraindications, Warnings. Nursing Mothers—See Warnings.

Pregnancy—Category X. See Contraindications, Warnings.

Nursing Mothers—See Warnings.

Adverse Reactions—An increased risk of these serious adverse reactions has been associated with use of OC's (si Warnings): thrombophlebitis, pulmonary embolism, coronary thrombosis, cerebral thrombosis, cerebral hemorhage. hypertension, gallbladder disease, benigin hepatomas, congenital anomalies. There is evidence of an association between the following conditions and use of OC's although additional confirmatory studies are needed: mesenteric thrombosis, neuro-ocular legisions, e.g., retinal thrombosis and optic neurits.

The following adverse reactions have been reported in patients on OC's and are believed to be drug-related. Nause and/of vomiting, usually the most common adverse reactions, occur in approximately 10 percent or less of patient during the first cycle. Other reactions, as a general rule, are seen much less frequently or only occasionally.

Gastrombestinal symptoms (such as abdominal cramps and bloating), breakthrough bleeding, spotting, change in mensitual flow, dysmenorrhea, amenorrhea during and after treatment, temporary infertility after discontinuance of treatment, edema; chloasma or melasma which may persist, breast changes: tenderness, enlargement, and secretion; change in weight (increase or decrease); change in cervical erosion and cervical secretion; possible diminution in lactation when given immediately postpartum; cholestatic jaundice; migraine; increase in size of uterine leiomyomata; rash (allergic); mental depression; reduced tolerance to carbohydrates; vaginal candidiasis; change in corneal curvature (steepening), intolerance to contact lenses. The following adverse reactions have been reported users of OC's, and the association has been neither confirmed nor refuted; premenstrual-like syndrome, cataracts; changes in libido, chorea, changes in appetite, cystitis-like syndrome, headache, nervousness, dizziness, hirsutism, loss of scalp hair, erythema multiforme, erythema nodosum, hemorrhagic c

For full details on dosage and administration see prescribing information in package insert.



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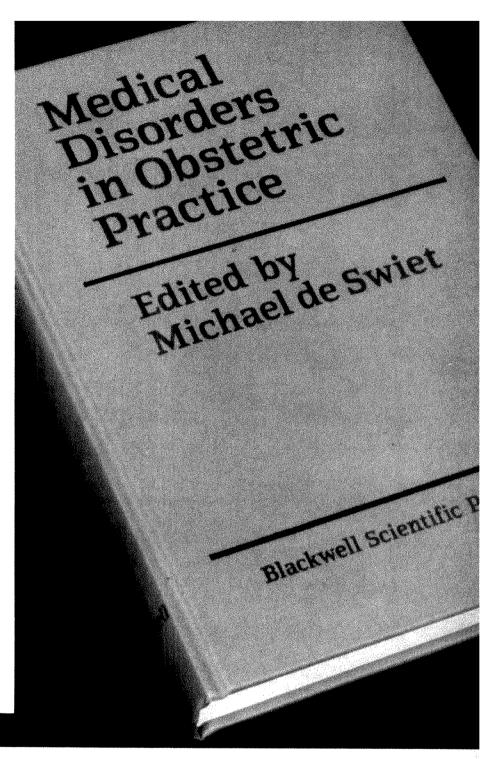
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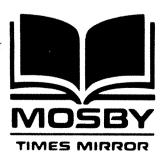
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The survival of very low-birth weight infants by level of hospital of birth: A population study of perinatal systems in four states

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This study estimates differentials in survival among very low—birth weight infants according to hospital of birth, and seeks to determine importance of birth at high-technology centers versus birth at other urban or rural hospitals. Data from four states for 1978 and 1979 were used to estimate survival curves for the first 24 hours of life by type of hospital at birth, birth weight, and race. Significant (p <0.0001) differences in survival by type of hospital for both races at birth weights of 1000 to 1500 gm were observed. Smaller disparities were seen at birth weights of 750 to 1000 gm. Differentials in survival by hospital setting emerged in the first few hours after birth, underscoring the effectiveness of neonatal intensive care units in reducing infant mortality and the importance of maternal transport. Differentials persisted throughout the neonatal and costneonatal periods, although differences were attenuated. Prenatal assessment and provider and institutional cooperation can contribute to lowered mortality for high-risk infants and mothers. (AM J OBSTET GYNECOL 1985;152:517-24.)

Key words: Very low birth weight, neonatal mortality, neonatal intensive care, survival curves

Population-based data in the United States demonstrate that birth weight-specific neonatal mortality has been decreasing in recent decades, with the most dramatic successes being reported in the survival of very low-birth weight infants (<1501 gm). Data from a variety of regional centers providing neonatal intensive care indicate that the neonatal survival of these infants, that is, survival to 28 days after birth, has increased from 38% in the 1960s to 50% in the early 1970s and 69% in the late 1970s.1 However, the benefits of advances in technology are not being provided equitably to all very low-birth weight infants. For example, in 1976 to 1978, in the New York metropolitan area, only 34% of very low-birth weight infants were born at the centers providing such care.2 In Iowa, in 1978, only 22% of very low-birth weight infants were born in that state's one regional center.3 There are many possible explanations for this lack of successful development of regionalized perinatal systems. These include barriers

at the individual, provider, and institutional levels. In addition, there is considerable discussion of the cost-benefits of the new technology, particularly for infants of 500 to 1000 gm.⁴

This study addresses three questions relevant to the discussion of what constitutes optimal use of neonatal intensive care technology for very low-birth weight infants. First, does survival of these infants vary by the technologic sophistication of the hospital of birth, and if so, how quickly after birth do these differences emerge? Second, do differences persist throughout the first year of life, or is mortality only postponed by access to advanced technology? Third, is neonatal intensive care technology equally beneficial for infants of different races and birth weights? We measured survival by single hours following birth to identify the times of greatest risk differential and to note the speed with which the differentials emerge. Data from four states from 2 years were used, affording us the ability to analyze various subgroups of infants and to observe statewide systems of care.

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Methods

Linked birth and death certificate data. Linked birth and death certificate data from Louisiana, Ohio, Tennessee, and Washington, from 1978 and 1979, were used in the study. A total population of 46,089 single and 7859 plural births, with infants <2501 gm in birth weight, were available for analysis; 9021 of these infants

Table I. Hospital of birth of infants	~1F00 1 · 1 /	1 '1'	1	1000 11000
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Table 1. HOSDIIAI OI DII III OI IIII AIIIS	~1300 gm. by race, urban/rura	i i coide nec.	and state.	1370 4110 1373

n		*	State		
Race/residence and hospital level at birth	Louisiana	Ohio	Tennessee .	Washington	
White, urban (n)	536	2059	643	201	
Level III (%)	27	26	40	42	
Other urban (%)	' 71	72	56	47	
Rural (%)	1	2	4	11	
Black, urban (n)	894	1081	576	10	
Level III (%)	59	35	67	40	
Other urban (%)	41	65	33	60	
Rural·(%)	• –	1	· <u>-</u>	-	
White, rural (n)	342	543	510	644	
Level III (%)	. 14	13	38	44	
Other urban (%)	34	10	16	43	
Rural (%)	52	77	47	13	
Black, rural (n)	460	116	186	56	
Level III (%)	31	- 11	45	61	
Other urban (%)	, 28	. 12	10	39	
Rural (%)	41	77	45	. .	

weighed <1501 gm. Comparable data available for analysis across the four states included date of infant's birth and death, time of death, hospital of birth, birth weight, race, date of mother's last menstrual period, and county of residence. Complete data on all items except last menstrual period were available for 99% of the births in this sample; data on last menstrual period were available for 88% of the records. Some codes were not available across states in these linked files; for example, birth weights of <1000 gm were coded as one category in Ohio and left as a continuous variable in other states. None of these omissions seriously affected the analysis.

Level of hospital of birth. There are no uniform criteria for neonatal intensive care units. The Committee on Perinatal Health, formed by representatives of the American College of Obstetricians and Gynecologists, the American Medical Association, the American Academy of Pediatrics, and the American Academy of Family Physicians in 1976, produced guidelines for the regionalization of maternal and perinatal health services that include criteria for level I, II, and III hospitals. They write, "These guidelines are for planning only and are not to be taken as standards of care. They have not been written as absolutes or regulations to be uniformly applied throughout the country." The "Standards and Recommendations for Hospital Care of Newborn Infants" of the American Academy of Pediatrics includes a similar caveat. Some states and other jurisdictions have designated criteria to use in evaluating maternity services; the Bureau of Maternity Services and Family Planning of the New York City Health Department, for example, evaluated each hospital's perinatal service and assigned a level between 1976 and 1978.2 However, none of the states in this study has established legally enforceable standards.

For this study we have neither a set of standards for the hospitals in each state nor the capability of scoring the hospitals by level independently. Instead we have had to rely on the consensus in each state on what the level III neonatal intensive care units are. All hospitals not designated level III are grouped together as level I/II urban, within a Standard Metropolitan Statistical Area (SMSA), or level I/II rural, outside an SMSA. To identify the level III neonatal intensive care units, we have relied on information provided to us by the state health departments, heads of state university perinatal units, and others working in maternal and infant care. In no case did we encounter disagreement among our informants. Of the four states, the designations were least clear in Washington, where a number of hospitals were identified as level II+. Such hospitals might be ones lacking capability only in some kinds of specialized pediatric surgery or might be so far from the University Hospital in Seattle that they act as local level III referral centers, although from the point of view of the university they are not quite level III. Another gray area is where a hospital at a distance from Seattle might have had a neonatalogist at some times and not at others. In sum, our definition of a level III neonatal intensive care unit is very conservative. Given that it is very difficult to identify even these highest level centers, we made no attempt whatsoever to distinguish between level I and level II perinatal services.

Data analysis. Infant mortality rates and survival curves are estimated for race and birth weight—specific groups, as well as for types of hospital of birth as described above. Race and birth weight are thus controlled in these analyses because these variables are major predictors of infant mortality, and their distribution varies substantially among hospitals. In our analyses, we categorize births by 500 gm weight categories. An

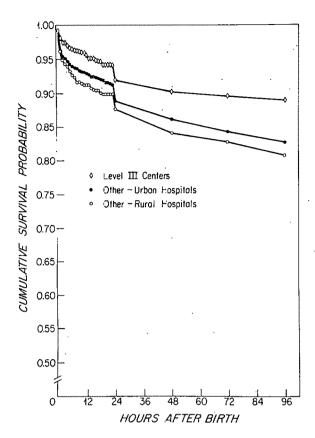


Fig. 1. Cumulative survival of white infants, 1000 to 1500 gm. first 96 hours after birth in four states, 1978 to 1979.

empirical analysis of the relationship between neonatal mortality and birth weight demonstrated that finer weight groupings add little explanatory power, except for the 500 to 1000 gm range, which is broken into two 250 gm intervals. Two other analytic techniques are used to control for additional variables that might otherwise bias estimates of the relationships between hospital type at birth and subsequent death: multivariate logistic regression and the proportional hazards model.6 Further variables controlled in these analyses include length of gestation and whether the birth was plural or singular. A previous analysis of institutional differences in infant mortality among low-birth weight infants found that once birth weight, gestation, race, and sex were controlled, other potentially confounding variables exerted little statistically significant influence on neonatal mortality.7 Survival curves are estimated with the Statistical Package for the Social Sciences survival program.8

Results

Selection of hospital of birth. Data from the four states under study indicated wide variation in the extent to which infants of very low birth weight are born in specialized centers (Table I). Only a fraction of the very low-birth weight births in these states took place in

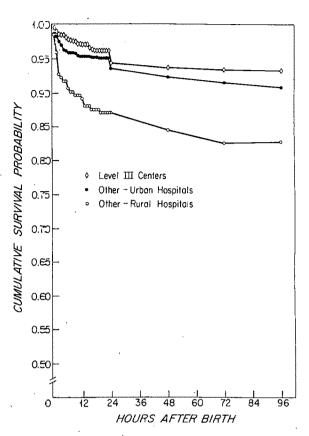


Fig. 2. Dumulative survival of black infants, 1000 to 1500 gm, first 96 hours after birth in four states, 1978 to 1979.

specialized centers. The smallest proportion of very low-birth weight infants born in level III centers occurrec among whites in Louisiana and Ohio; overall, 23% of white very low-birth weight infants were born in regional centers in these states. Black infants, in general, had better access to these specialized services. In Washington and Tennessee more than 50% of the black very low-birth weight infants were born in these centers. The lowest percentages of births occurring in regionalized centers, as one would expect, were to residents of rural areas, but here again, black infants were more likely than white ones to be born in specialized

Estimating the impact that the place of birth had on the survival chances of such high-risk infants was constrained by the obvious fact that pregnant mothers and hence births were not randomly assigned to the various types of institutions. Some high-risk fetuses were identified during the prenatal period, and maternal transfers took place with the fetus at risk in utero. Maternal transfers were also possible during premature labor. Thus some hospitals should have been delivering more very low-birth weight and more high-risk infants than others. Other data not shown in Table I indicate that this sort of selection was operating in each of these states. The proportion of 1000 to 1500 gm births taking

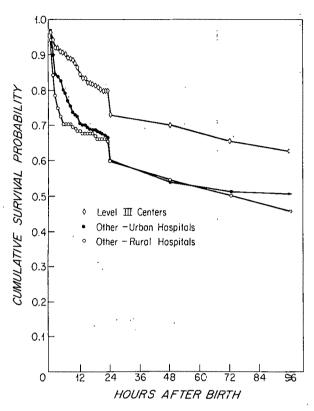


Fig. 3. Cumulative survival of white infants, 750 to 1000 gm, first 96 hours after birth in four states, 1978 to 1979.

place in level III centers was larger than the proport on of 2500+ gm births taking place in these centers. In addition, transfers could take place soon after delivery. High-risk infants born in a level I hospital who were strong enough to survive the first hazardous hours after birth could soon be enjoying all the facilities of a level III hospital. Our analysis therefore leads to minimal estimates of the impact of neonatal intensive care on the survival of all very low-birth weight infants.

Survival by hour after birth. We took two approaches to estimating the effect of place of birth on subsequent survival in the present analysis. We first examined the time period immediately following birth (the first 96 hours) in order to analyze closely the influences on mortality that were related to the hospital of birth. Differences in survival that emerged during this period for specific groups of infants at risk could be reasonably attributed, we believe, to the level of technology and skills available at the hospital of birth. We then looked at the survival experience of infants throughout their first year after birth in order to see the longer-term impact of differences in the perinatal care received and to test the hypothesis that specialized care may simply postpone the death of high-risk infants to the postneonatal period.9

Survival curves by hospital of birth were estimated for 10 different birth weight/race groupings. No sta-

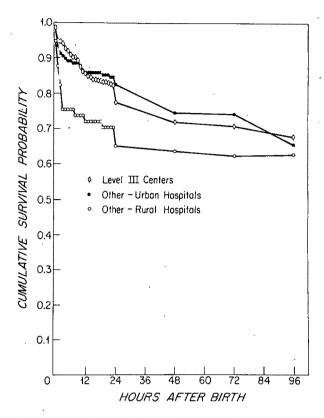


Fig. 4. Cumulative survival of black infants, 750 to 1000 gm, first 96 hours after birth in four states, 1978 to 1979.

tistically significant differences in survival were noted for either black or white infants of 2000 to 2500 or 1500 to 2000 gm. In contrast, significant differentials in early survival did appear among infants of <1500 gm at birth. Figs. 1 and 2 plot the 96-hour cumulative survival of 1000 to 1500 gm white and black infants by their hospital of birth. In these graphs, the differentials in survival between births occurring in the different hospital types emerged mainly during the first few hours after birth. The curves were relatively smooth, with the exception of a sharp drop at 12 hours. Apparently, a number of deaths occurring around this point have been rounded off to 12 hours. The curves did not cross over time, however, and we did not consider this a problem.

The survival of white and black infants of 1000 to 1500 grn born in level III centers was significantly greater than that of infants born in rural hospitals (p < 0.0001). White infants born in level III centers also experienced significantly greater 4-day survival than white infants born in other urban hospitals (p = 0.0001), although the same was not true for black infants (p = 0.12). In all these comparisons, however, survival among infants born in the level III centers was highest.

Infants <1000 gm born in level III centers also experienced significantly better survival than infants born

Table II. Mortality of very low-birth weight infants by level of hospital of birth and race in four states, 1978 and 1979

	No. of deaths/1000 live births						
	Early neonatal death (0-4 days)	Neonatal death (0-28 days)	Postneonatal death (28 days–1 yr)	Infant death (birth–1 yr)			
750-1000 gm*			· ·				
White infants							
Level III (226)	358	486	58	544			
Other urban (296)	490†	588‡	44	632‡			
Rural (135)	519†	608‡	7‡	615†			
Black infants		· •	•	,			
Level III (289)	318	481	42	522			
Other urban (146)	315	486	48	534			
Rural (57)	386	404	88	491			
1000-1500 gm							
White infants							
Level III (1020)	113	162	30	192			
Other urban (1557)	171§	232§	31	263§			
Rural (543)	190§	250§	26	276§			
Black infants							
Level III (847)	73	111	32	143			
Other urban (718)	95	142	28	170			
/ Rural (189)	180§	233§	21	2548			

Numbers in parentheses are counts of live births.

in either other urban or rural hospitals. A further analysis by 250 gm intervals revealed no significant differentials in survival among the 500 to 750 gm births but substantial differentials by hospital group among white and black births of 750 to 1000 gm. These survival curves are plotted as Figs. 3 and 4. (Data from Ohio have been omitted from these figures because 250 gm breakdowns of the Ohio data were not possible.) Among the white births, the differences between the survival of infants born in level III versus either of the other hospital groups were significant at p < 0.001. Among the black births, the differences were not statistically significant (p = 0.84 and p = 0.10). The black 750 to 1000 gm infants born in rural hospitals experienced initially much greater mortality, but this difference was almost gone by the end of 4 days. The samples of black births in rural hospitals was relatively small, however, and thus statistical insignificance could be expected.

These curves illustrate the high risk of mortality experienced during the first few hours after birth and thus indicate the constraints under which the delivery of optimal perinatal care operates. Any deterioration which takes place early in the life of a high-risk newborn infant is probably an additional risk factor later on, no matter what level and expertise of care are subsequently available. Within the first few hours, in Figs. 1 to 4, curves for both black and white infants born in the level III centers diverged sharply from the curves of infants

not born in these centers. The largest differentials appeared in the contrasts between infants born in level III centers and those born in rural hospitals. A look at the hazard rates estimated from these survival data confirms the substantial differences in risk experienced by infants during the first few hours after birth. White infants of 1000 to 1500 gm experienced mortality of 0.9% per hour during the first 3 hours if the birth occurred in a level III center; white infants of similar birth weight born in rural hospitals experienced mortality of 1.8% per hour during this time period. Among black infants weighing 1000 to 1500 gm, the hazard rate was 0.5% per hour if the birth occurred in a level III center and 2.6% per hour if in a rural hospital.

Survival of black infants versus white infants. As we have already noted, there were substantial differences in birth weight-specific survival between white and black infants. Many of the differentials in early survival by race were of a magnitude similar to the differentials noted by hospital of birth. In general, for all birth weight categories, survival during the first 96 hours was greater among black than among white infants at the same level of hospital care. Racial differentials in birth weight-specific survival have long been noted10,11 and thus were not surprising. We could have combined these different curves in our analysis and statistically controlled for race, but we believed that the separate curves were useful descriptive data in their own right. Furthermore, some expected differentials

^{*}Data from Ohio not available; analysis of births <1000 gm from all four states, however, revealed very similar results.

[†]Difference between level III and this category significant at p < 0.01.

[‡]Difference between level III and this category significant at p < 0.05.

[§]Difference between level III and this category significant at p < 0.001.

Table III. Estimated relative risks of death by birth weight, level of hospital of birth, and race in four states, 1978 and 1979

	Estimated :elative risk							
	Early neonatal period (0-4 days)	Neonatal period (0-28 days)	Postneonatal period (28 days–1 yr)	Infancy (birth–1 yr				
750-1000 gm*.								
White infants	•							
Level III	1.00	1.00	1.00	1.00				
Other urban	1.36†	1.21‡	0.76	1.16‡				
Rural	1.45†	1.25‡	0.12‡	1.13†				
Black infants	•	•						
Level III	1.00'	1.00	1.00	1.00				
· Other urban	0.99	1.01	1.14	1.02				
Rural	1.21	0.84	2.10	0.94				
1000-1500 gm			•					
White infants	· · · · · · · · · · · · · · · · · · ·							
Level III	1.00	1.00	1.00	1.00				
Other urban	1.518	1.43§	1.03	1.37§				
Rural	1.68§	1.54§	0.87	1.448				
Black infants			****					
Level III	1.00	1.00	1.00	1.00				
Other urban	1.30	1.28	0.88	1.19				
Rural	2.47§	2.10§	0.66	1.78§				

^{*}Data from Ohio not available; analysis of births <1000 gm from all four states, however, revealed very similar results.

by race did not emerge as statistically significant. For example, among 750 to 1000 gm infants born in level III centers, no statistically significant differences in survival appeared (although survival of black infants was slightly higher). Among 1000 to 1500 gm births, black infants born in rural hospitals did not fare significantly better than white infants born in rural hospitals. Because of the differences in birth weight—specific survival by race, it was important in comparing outcomes among hospitals or geographic areas to control for race as well as for birth weight distribution.

Controlling for other variables. We have already mentioned other variables that have been related to infant survival independently of birth weight and race, such as gestation and plural or singular birth. When we analyzed the data after controlling for these variables we found that the differentials described above by hospital of birth remained essentially unchanged after these controls were introduced as co-variates into proportional hazards models. No differentials in survival for singular versus plural births were noted once birth weight was controlled. This was different from what Williams et al.12 found in California; further investigation of this question may be in order. Some differentials in survival did emerge among states, even after birth weight, race, and hospital type were controlled. These, however, were generally small.

Neonatal and postneonatal survival. The mortality of these 750 to 1000 gm infants and 1000 to 1500 gm

infants throughout their first year of life is summarized in Table II. The relative risks associated with the different types of hospitals are summarized in Table III. The largest relative risks appeared for infants of 1000 to 1500 gm. Generally small and inconsistent differentials appeared for 750 to 1000 gm births. The data in both of these tables indicated that the initial early differentials in risk of neonatal mortality by hospital of birth persisted throughout the first year of life. While some greater risks of postneonatal mortality appeared among infants born in level III centers, these did little to atteruate risks by hospital of birth which emerged during the first few days. Infants born in level III centers, in general, experienced the best survival, while infants born in the rural hospitals experienced the worst survival. In general, black infants had better survival rates than white infants of the same birth weight and hospital of birth.

Comment

We have demonstrated significant differentials in the survival of infants of similar race and birth weight, depending on their hospital of birth. These differentials appear among infants weighing 750 to 1500 gm at birth for both black and white infants (in only three states for 750 to 1000 gm births). The results are consistent with those of other studies of the survival of very low-birth weight infants, including studies that are institutionally based and those that are population

[†]Difference between level III and this category significant at p < 0.01.

[‡]Difference between level III and this category significant at p < 0.05.

[§]Difference between level III and this category significant at p < 0.001.

based.18 The neonatal survival rate of 500 to 1500 gm infants born in level III centers in the three states with these birth weight data was 68% for both black and white infants,* remarkably close to the 69% noted in a recent literature review of the experience of regionalized centers.1 The advantages in survival we demonstrated among infants born in level III centers versus those born in other hospitals located in metropolitan areas are also consistent with the results of two previous population-based studies.2,3 The relative risks associated with very low-birth weight infants born in the rural hospitals in this study were often higher than those reported for the urban hospitals and were substantially higher than those reported for births taking place in level III centers.

Our analysis of survival by hour following birth illustrates the rapidity with which these mortality differentials emerge. This corroborates the most recent work done by Paneth et al.14 The advantages of maternal as opposed to infant transport for very low-birth weight infants become obvious. Even if excellent infant transport is available in rural areas, the hours involved place very low-birth weight infants at risk. Our data show that the neonatal mortality risk differentials by hospital of birth are sustained throughout the first year of life and are reflected in infant mortality rates. While some deaths are merely postponed into the postneonatal period by birth at level III centers, more often death is prevented.

Finally, it needs to be emphasized that the numbers of handicapped very low-birth weight survivors is likely to be small. The infants whose cost-benefit status seems most questionable at this point are those weighing 800 to 1000 gm.4 These constitute about 0.4% of all live births in the United States. If we assume that 1-year survival is 37% and that the rate of handicaps among survivors is 40% (twice what Bennett et al.15 found in a recent study), the number of handicapped individuals that could be maximally attributed to improved care is still only 0.6/1000 live births. As long as any new technique used in the future is carefully evaluated, providing intensive services at the time of birth more equitably and to larger numbers of infants at risk should improve both the survival prospects and the chances for a healthy life for many children.

Our results also illustrate the difficulty of evaluating changing neonatal intensive care unit technologies because of the very different survival patterns observed for infants born in different level hospitals and for

*While the overall percentage of survival is similar for black and white infants, birth weight-specific survival for 500 to 750 and 1000 to 1500 gm is greater for black births than for white births. This differential is counterbalanced by the lighter birth weight distribution of the black infants.

infants of differing birth weight, gestation, and race. The differentials by race are large and indicate that black very low-birth weight infants have better survival than white infants of similar birth weight. Among infants of 1000 to 1500 gm born in level III centers in these four states, white infants appeared to experience neonatal mortality almost 50% greater than that of nonwhite infants. Adequate evaluation of neonatal intensive care units thus requires the estimation of separate rates by race.

Although there is no agreement on what would constitute the optimal system of perinatal care, it is clear from our study that very low-birth weight infants benefit greatly from being born in a level III perinatal center. Unless pregnant women are enrolled in a system of prenatal care that includes identifying high-risk pregnancies and premature labor, maternal transfers cannot easily take place. The key to improving the present system of care for very low-birth weight infants lies in improving prenatal care for all pregnant women. An added benefit of a comprehensive and universally accessible system of prenatal care is that good care combined with techniques for early detection and prevention of premature labor holds the promise of reducing the incidence of low birth weight and thereby the demand for high-cost, high-technology neonatal intensive care.16

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The Dublin randomized controlled trial of intrapartum fetal heart rate monitoring

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In a randomized controlled trial involving 12,964 women, a policy of continuous electronic intrapartum fetal heart monitoring was compared with an alternative policy of intermittent auscultation, both policies including an option to measure fetal scalp blood pH. Women allocated to ele-ronic fetal heart monitoring had shorter labors and received less analgesia. The caesarean delivery rate∈ were 2.4% for electronic fetal heart monitoring and 2.2% for intermittent auscultation but this small dif∈rence arose from the identification of nearly twice as many fetuses with low scalp pH (<7,20) in the electronic fetal heart monitoring group. The forceps delivery rate was 8.2% in the electronic fetal bart monitoring group compared with 6.3% in the intermittent auscultation group, and this excess w≡s explained by more instrumental deliveries prompted by fetal heart rate abnormalities. There were 14 stillbirths and neonatal deaths in each group, with a similar distribution of causes. There were no apparent differences in the rates of low Apgar scores, need for resuscitation, or transfer to the special care nursery. Cases of neonatal seizures and persistent abnormal neurological signs followed by survival wer≥ twice as frequent in the intermittent auscultation group, and this differential effect was related to dura3 on of labor. Follow-up at 1 year of babies who survived neonatal seizures revealed three clearly abnormal infants in each group. The implications of these findings for both theory and practice are discussed. (AM J OBSTET GYNECOL 1985;152:524-39.)

Key words: Fetal monitoring, labor, random allocation, delivery, newborn infant

During the past decade there have been dramatic changes in the methods used to assess the condition of

the fetas during labor. Intermittent auscultation has increasingly been displaced by continuous recording of the fetal heart rate. This widespread trend has been associated to a variable extent with the use of fetal scalp blood sampling to assess fetal acid-base status when the fetal heart rate pattern causes concern.

Conversy continues about the relative merits of these different methods of intrapartum fetal assessment. Although there is some evidence of a consensus that the use of the more intensive methods of intrapartum monitoring is appropriate when the fetus is deemed to be at high risk, there is no such agreement

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Reprint requests: Dr. Dermot MacDonald, National Maternity Hospital, Holles St., Dublin 2, Ireland. concerning the application of these methods for fetuses at average or low risk of adverse outcome.1

The striking variations that exist in both opinion and practice reflect the dearth of unbiased comparisons of alternative methods of intrapartum fetal assessment in randomized trials. Claims that electronic fetal heart rate monitoring prevents intrapartum stillbirth and neonatal death more effectively than intermittent auscultation have been based on methodologically weaker, observational studies.1,2

Intermittent auscultation has been compared with continuous electronic fetal monitoring (with or without an option to assess fetal acid-base status) in six randomized trials so far. Three of these trials3-5 have compared intermittent auscultation with continuous fetal heart monitoring in women at relatively high perinatal risk, defined variously. Participants in the other three trials^{6,7} (Neldam S, personal communication) were at relatively low perinatal risk, again, defined variously.

All these trials had less than 500 subjects in each treatment group. Because of this, it would not be expected that any one trial on its own would have detected differences in the effects of alternative methods of monitoring on relatively unambiguous measures of adverse neonatal outcome unless these effects had been very dramatic. All of the trials suggest that electronic fetal heart monitoring can increase the cesarean delivery rate, particularly if no attempt is made to assess fetal acid-base status. Based on pooled within-center differences8 in these six trials, the risk of cesarean delivery associated with electronic fetal heart monitoring used on its own is increased 2.7 times (95% confidence limit 1.9 to 3.8). This increase appears to be smaller (1.9, 95% confidence limit 1.6 to 2.2) if fetal scalp blood sampling has been used as an adjunct to electronic fetal heart monitoring.

In only one trial4 was there evidence that this increased cesarean delivery rate led to improved fetal outcome. The results suggested that electronic fetal heart monitoring used with scalp sampling might reduce various forms of abnormal neonatal neurological parameters. Furthermore, a pooled analysis of all the trials conducted by Chalmers9 revealed a distribution of neonatal seizures which was unlikely to have occurred by chance. There were fewer seizures among babies who had been monitored by electronic fetal heart monitoring used in conjunction with fetal acid-base assessment when indicated. There was little evidence of such an effect when electronic fetal heart monitoring had been used alone. These observations are important because seizures are often associated with serious sequelae. About a quarter of all infants with seizures die in early infancy, and a similar proportion survive with serious disabilities.10

It was against the background of these findings

and the persistent calls for further larger-scale trials to help resolve the controversy about the place of the more intensive methods of intrapartum fetal monitoring1.3-5,7.9 that, in 1980, the possibility of a large randomized trial was considered at both the National Maternity Hospital, Dublin, and the National Perinatal Epideniology Unit in Oxford.

Since 1975, selection of cases for intensive intrapartum fetal monitoring at the National Maternity Hospital has been based on explicit criteria.11 It is important to emphasize that only women who are considered to be in labor are admitted to the labor ward and that each woman in labor has a personal nurse.11 The amniotic fluid is inspected within an hour of admission, by amniotomy if necessary. In some 5% of cases there is moderate or dense meconium or no fluid is obtained. In these cases, the fetus is considered to be at special risk and fetal scalp blood pH is measured. If the level is <7.25, the baby is delivered as soon as possible. The others receive continuous electronic fetal heart monitoring. Prior to this study, in the remaining 95% of cases in which clear fluid was observed, the fetus was supervised by auscultation at regular intervals and by scalp blood sampling as indicated.11 Blood sampling was also performed when meconium developed later and when the duration of labor exceeded 8 hours. Delivery was expedited if the pH estimation was <7.25.

A trial was mounted to compare this latter policy with the most promising alternative policy-continuous fetal heart rate monitoring by means of a scalp electrode, with scalp blood sampling when indicated—in the 95% of cases with a normal volume of clear or only lightly meconium-stained fluid. This paper describes the short-term clinical and biologic effects of the two policies on mothers and babies. A detailed report of the background, planning, execution, and short-term results of the trial, containing a full bibliography, is available.18 Other articles will describe the fetal heart rate patterns and babies with abnormal neonatal neurological signs more extensively than this one. Further publications will report maternal and staff views, the economic implications of the two policies, and longer-term biologic effects in childhood.

Material and methods

Hypotheses and sample size. The principal measures of adverse outcome for babies (excluding those with congenital abnormalities causing death or abnormal neurological characteristics) were (with anticipated frequencies calculated from published annual reports of the hospital): intrapartum stillbirths, 1/1000; neonatal deaths, 2/1000; neonatal seizures in survivors, 3/ 1000; and other severe abnormal neurological characteristics as defined below, 4/1000. The anticipated trial size of 10,000 had an 80% chance (power) of ob-

servation of a statistically significant difference at the 5% level if these adverse outcomes in combination were actually reduced by half, from 10/1000 to 5/1000, through a policy of more intensive fetal heart mcnitoring. The study would have carried a 50% power if the actual reduction were by a third, to 6.7/1000. The size of these hypothesized differences was based on claims made on the basis of observational studies =. 13 Following the interim analysis (when only 4000 caes had been recruited, see below) it was decided that atter 10,000 cases had been entered to test these hypothe es, recruitment would be extended to 13,000 to assess the effect of electronic heart rate monitoring on the most unambiguous adverse neonatal outcomes, deaths and seizures. A trial of this size has a 75% power if, in truth, the total number of babies who die or who survive seizures is reduced by half, from 6/1000 to 3/1000, and a 50% power if the overall rate of seizures is redued by half, from 4/1000 to 2/1000.

Two stratified analyses were prespecified in the trial protocol. The first, by predefined "risk status" (see Eligible Cases, below), was to assess whether the differential effects of the two policies were greater in he high-risk group. The second stratified analysis, by tene interval between entry to the trial and delivery (cne stratum including mothers who were delivered witin an hour of entry and the other stratum including all those for whom the interval was greater than an hour), was chosen for two reasons: first, to assess whether fital surveillance was suboptimal while electronic fetal heart monitoring was being organized and before a satistictory tracing was available, and second, to select a group in which a higher proportion of women were mcnitored with the instrument to which they had been allocated.

Primarily for practical reasons, it was decided to limit the data collection on umbilical venous acid-base status following delivery to 1000 consecutive babies. A comparison based on this sample size had a 90% chance of finding a statistically significant difference (two-taied $\alpha=0.05$) if the true difference in the mean umbilical venous pH of the two groups was 0.015 and a 50% power if the true difference was 0.01.

Study population. Entry into the trial began on March 31, 1981, and ceased on April 10, 1983. Durng this period 17,381 mothers were delivered at the Jational Maternity Hospital.

Exclusions. Of these 17,381 women, 4356 (25.7%) were ineligible for entry to the trial. A total of £71 (3.3%) were delivered by elective cesarean section ≡nd 109 (0.6%) suffered a fetal death prior to the onse of labor. Thus 16,701 women had a live fetus at the ⊃eginning of labor. Of these women, 2836 (17.0%) w≡re delivered so rapidly after arrival in the labor ward (\$50%)

within I hour of admission) that it was not possible to examine the quantity and meconium status of the amniotic fluid and thereby assess whether they were eligible for entry into the trial. [One intrapartum and nine neonatal deaths of normally formed babies (overall death rate 3.5/1000) subsequently occurred in this group.] Forty-eight (0.3% of the women with a live fetus at the beginning of labor were ineligible for entry into the trial either because the pregnancy had not reached 28 completed weeks of gestation or because a gross fetal abnormality had been recognized prior to labor. [There were subsequently 22 intrapartum and 15 neonatal deaths in this group (overall death rate 771/1000).] Amniotic fluid was formally inspected in 13,817 women who were potentially eligible for the trial. In 792 women (5.7%), there was either no fluid, undiluted thick meconium, or a heavy suspension of meconium in a reasonable volume of fluid. These cases were managed as described above. [There were subsequently three intrapartum and six neonatal deaths of normally formed babies in this group at special risk (11.4/1000).]

Eligible cases. There remained 13,025 women eligible for inclusion in the trial because (1) they had a live fetus of at least 28 weeks' gestation with no evidence of gross abnormality, (2) a diagnosis of labor had been made, and (3) amniotic fluid without significant meconium staining had been positively demonstrated either at spontaneous rupture of the membranes or at early amniotomy. Of these 13,025 eligible women 12,964 (99.5%] were entered into the trial and these women were delivered of 13,084 babies. The trial therefore included most babies of more than 28 weeks' gestation and most mothers with medical complications of pregnancy. A total of 22.5% of cases met one or more of the following criteria which were predefined for the purpose of this study as signifying increased risk: maternal age of 40 years or more, diabetes mellitus, preeclampsia, chronic hypertension, renal disease, cardiac disease, previous stillbirth or neonatal death, previous child with neurological abnormality, previous low-birth weight baby, bleeding in pregnancy requiring admission to the hospital after the first trimester, induction of labor for pregnancy of more than 42 completed weeks' gestation, multiple pregnancy, breech presentation in labor, and gestaticnal age less than 34 completed weeks.

Rancom allocation. Immediately after eligibility had been confirmed by examination of the amniotic fluid, random allocation either to continuous electronic fetal monitoring and fetal scalp blood sampling when indicated or to intermittent auscultation and scalp sampling when indicated as outlined above was achieved by opening the next envelope in a series of serially numbered, sealed, opaque envelopes. Because of the large size of the trial prognostic stratification was not used.

Treatment schedules

Electronic fetal heart monitoring group. As soon as possible after randomization, an electrode (Surgicraft Copeland) was applied to the fetal scalp and an external tocodynamometer applied by the labor ward resident. (A maximum of two attempts were made to attach a scalp electrode during a single vaginal examination to avoid maternal discomfort.) After attachment of the scalp electrode, the fetal heart rate was validated by auscultation to check that the monitor was functioning correctly. When it proved impossible to obtain a satisfactory signal from a scalp electrode, an external transducer was used. If a woman's personal nurse-midwife was concerned about the electronic fetal heart monitoring tracing, she first checked the fetal heart by auscultation and then informed the nurse-midwife in charge of the labor ward. If she, in turn, also considered the tracing to be abnormal (see below), an experienced obstetrician was summoned.

The following fetal heart rate patterns (adapted from the classifications proposed by Haverkamp et al.3 and Gillmer and Beard¹⁴) were considered to be suspicious or ominous: marked tachycardia or bradycardia, moderate tachycardia or bradycardia with reduced variability, minimal variability (absent beat-to-beat variation, flat tracing), late deceleration pattern, moderate and severe variable deceleration patterns and other confusing patterns with varying baselines which could not be clearly interpreted. If any of these patterns had been present for at least 10 minutes and had not responded to conservative measures (changing maternal position, adjusting transducers, etc.), clinical action was to be taken. In the first stage of labor, fetal scalp blood pH was to be measured. In the second stage of labor, delivery was to be undertaken immediately.

If a scalp blood pH estimation was <7.20, delivery was effected as soon as possible. If the pH was between 7.20 and 7.25 and the fetal heart pattern remained suspicious or ominous, delivery was also effected as soon as possible; if the fetal heart rate reverted to a pattern which was neither suspicious nor ominous, however, the situation was managed expectantly. If the pH was >7.25 and the tracing remained suspicious or ominous, scalp blood pH was remeasured between half an hour and an hour later; if the fetal heart reverted to a pattern that was neither suspicious nor ominous, no further action was required unless the pattern again became abnormal.

Throughout the duration of the trial all tracings were reviewed by a single experienced observer (P. B.) who was "blinded" to neonatal outcome following delivery. Based on fetal heart rate patterns that persisted for at least 10 minutes, each tracing was given a first-stage and second-stage classification. The primary classifi-

cation was into patterns which the observer considered should or should not have prompted clinical action. Within these broad groups the type of pattern was then described. If it was considered that a pattern should have prompted action the clinical management as recorded on the tracing was reviewed. Some abnormal patterns were judged to have resolved spontaneously; some responded to conservative measures such as a change in maternal position; some prompted "appropriate action." This usually meant that fetal blood had been sampled during the first stage of labor or that immediate delivery had been effected in the second stage. In the remainder it was judged that appropriate action had either been delayed or not been taken. The three objectives of this review were: (1) to audit tracing interpretation and management and thereby ensure that the quality of continuous electronic fetal heart monitoring remained high, (2) to describe the fetal population in terms of the prevalence of fetal heart rate tracing patterns, and (3) to describe compliance with the electronic fetal heart monitoring policy.

Auscultation group. Women allocated to auscultation were managed according to the hospital's existing policy for cases with amniotic fluid free of heavy meconium staining. Following a contraction, the fetal heart was auscultated with a Pinard stethoscope for 60 seconds, at least every 15 minutes in the first stage and during every interval between contractions in the second stage of labor. If difficulty was encountered in detecting the fetal heart rate by auscultation, intermittent Doppler ultrasound was used.

If the fetal heart rate was >160 or <100 bpm during three contractions and the abnormality failed to respond to conservative measures (for instance, change in maternal posture or treatment of maternal pyrexia), either the scalp pH was estimated and a scalp clip attached or delivery was expedited, again, depending on the stage of labor.

Compliance with this policy was assessed in 1000 randomly selected cases managed by intermittent auscultation. Cases were identified in which the fetal heart rate had not been recorded as described in the protocol or where an abnormality in the rate had been recorded but with no documentation that appropriate action had been taken.

Measurement of adverse fetal outcomes

Deaths. All intrapartum deaths and deaths within 28 days of delivery (neonatal deaths) were examined at necropsy by a pathologist who was unaware of the method of monitoring to which the case had been allocated. Babies who died in the late neonatal period after discharge from the National Maternity Hospital were identified by the Central Statistics Office in Dublin. The system used to classify the deaths was based

on that proposed by Wigglesworth¹⁵ with the additional category of "birth trauma." Each case was first classified by primary cause of death. Those cases in which the primary cause was not considered to be "asphyxial conditions developing during labor" were then reviewed to assess whether such conditions could have contributed to the death.

Neurological abnormalities. One neonatologist (M. S.-P.) was responsible for neonatal neurological assessments throughout the duration of the trial. These were made without knowledge of the method of monitoring to which the case had been allocated (unlike spiral e.ectrodes, the Surgicraft Copeland electrodes, which were used throughout the trial, leave no obvious mark on the scalp). Babies were deemed to have had neonatal seizures if the neonatologist felt that the evidence was that of a seizure of the following types: generalized tonic, multifocal clonic, focal clonic, or myoclonic. Infants with "subtle seizure activity"16 or "jitteriness" were not included in this group. On rare occasions, when documented evidence was doubtful, the events were discussed with the observers present at the time before a final decision was reached.

During recruitment of the first 10,000 subjects, serial standardized assessments were made on all babies admitted to the special care baby unit and any baby on the lying-in wards about whom there was cause for concern. This latter group was identified in three main ways. First, regular visits were made by the neonatologist to the postnatal wards to search for sick babies. Second, any neonate with problems was referred directly to the neonatologist by the nurse in charge. Last, each baby had a routine examination by one of the pediatric hospital staff during the first week of life and, again, any baby with abnormality was referred to the neonatologist.

All babies identified in any of these ways were examined within 48 hours of birth with the method described by Dubowitz et al.¹⁷ They were reexamined after 72 hours of age, at 7 days after delivery (if still in the hospital), and at discharge. At each examination an overall assessment was made of tone and movement, reflexes, and neurological behavior. On the basis of these examinations, three further, mutually exclusive groups of babies with abnormal neurological characteristics were identified: first, those with simultaneous abnormalities of both tone and reflexes¹⁸; second, those with other neurological abnormalities that persisted 1 week after birth; and third, those with other, transient neurological abnormalities that had resolved by 7 days after delivery.

During recruitment of the final 3000 subjects, this protocol was simplified and the neonatologist identified only babies who had seizures in the neonatal period.

Apgar score. An Apgar score was recorded at 1 and 5 minutes after delivery.

Umbi ical venous blood pH. Collection of umbilical venous blood samples was limited to a 2-month period, December, 1981, and January, 1982. It was anticipated that this would generate pH estimations on more than 1000 trial babies, the number prespecified in the protocol (see section on sample size). A 15 cm length of umbilical cord was "double clamped" at birth and 3 ml of vencus blood was aspirated anaerobically into a heparinized polyethylene syringe (Sarstedt Monovette), which was capped and placed on a cold tray in a 4° C refrigerator. The sample was then analyzed on the IL System 1302 (Instrumentation Laboratories, Cheshire, England) blood gas analyzer. The median interval between delivery and analysis was 18 minutes and 85% of samples were analyzed within 60 minutes of delivery.

Pedictric follow-up. Babies surviving neonatal seizures cr other simultaneous abnormalities of tone and reflexes¹⁸ have been followed up for at least 1 year. They have been seen by one of two senior pediatricians who had not been directly involved in the trial and who were "blinded" to the trial allocation. Based on the examination system described by Sheridan, a general assessment has been made of posture and large movements, vision and fine movements, hearing, speech, and social behavior.

Statistical methods. Discrete variables are presented as cross-tabulations; continuous variables are presented as means \pm SDs or SEs or as medians with a measure of range. The χ^2 (with the Yates correction) and t tests of statistical significance have been used, as appropriate.

Ethical aspects

Consent. As first proposed by Zelen, 20 women allocated to the experimental policy (electronic fetal heart monitoring) were asked if they would consent to having their babies monitored by electronic fetal heart monitoring. No formal consent was sought from women allocated to the normal hospital policy (intermittent auscultation). The reasons for some women's rejection of electronic fetal heart monitoring have been reviewed and will be reported elsewhere. To avoid selection bias, however, these few women have been retained in their allocated group in the analyses presented here.

Possibility of early termination of trial. After approximately 4000 cases had been recruited (twice the number then available in all previous trials combined), an interim analysis was conducted by the two investigators (A. G., I. C.) who were not directly involved in the clinical management of the trial. No formal statistical tests were performed.² An obstetrician, a pediatrician, and a medical statistician, all of whom were independent of the study and of the National Maternity Hos-

Table I. Descriptive characteristics

	Electronic fe	tal menitoring	Intermittent auscultation	
Characteristic	No.	%	No.	%
Mothers**				
Maternal age (yr)				
<20	326	. 6.5	359	7.2
20-34	4147	83.2	4087	81.7
35+	491	9.8	530	10.6
NK	23	0.5	23	0.5
Marital status				*
Married	4479	89.8	4490	89.8
Other	492	9.9	488	9.8
NK	16	0.3	21	0.4
Ethnic origin	10	0.5	4.1	0.1
Caucasian	4958	99.4	4965	99.3
Other	14	0.3	17	0.3
NK.	15	0.3	19	0.3
Sociceconomic group of husband	13	0.5	1.0	0.1
1 and 2	2173	43.6	2258	45.2
3	717	14.4	722	14.4
4 and 5	1583	31.7	1521	30.4
Unmarried	482	9.7	470	9.4
Other	9	0.2	7	0.1
NK.	23	0.5	21	0.1
Parity at entry	23	0.5	2.1	01
0	2015	40.4	1964	39.3
1-3	2557	51.3	2601	52.0
4+	399	8.0	413	8.3
NK.	16	0.3	21	0.4
Induction of labor	434	8.7	475	9.5
High risk‡	1106	22.2	1137	22.7
Babies††	1100	44.4	1137	44.1
Gestational age (wk)				
28-33	31	0.6	24	0.5
34-36	125	2.5	109	2.2
37-41	4245	84.3	4232	83.7
42+	609	12.1	666	13.2
NK	26	0.5		0.5
	20	0.5	28	0.5
Birth weight (gm) <1500	5	0.1	9	Δ.
	119		3	0.1
1500-2499	3962	2.4	124	2.4
2500-3999		78.7	3969	78.5
4000+	926	18.4	934	18.5
NK	24	0.5	28	0.5

NK = Not known.

‡See definition in text.

pital, then compared the experience of the two groups (without the identity of either group being made available). They were asked to state whether there were differences in the frequency of either serious adverse fetal outcomes or cesarean sections which would lead them to recommend early termination of the trial. They were unanimous in recommending that the trial should not be terminated prematurely. No other person and, in particular, no one at the National Maternity Hospital was given any information about this interim analysis.

A total of 12,964 women entered the trial, of whom 6474 were allocated to electronic fetal heart monitoring and 6490 to intermittent auscultation. Analyses based on these women are marked with a single asterisk. These women were delivered of a total of 13,084 babies, 6530 in the electronic fetal heart monitoring group and 6554 in the intermittent auscultation group. Analyses based on these babies are marked with a dagger. In the first phase of the trial (during which more detailed data were collected) 9996 women entered the trial, 4987 in the electronic fetal heart monitoring group and 4999 in the intermittent auscultation group. Analyses based on these women are marked with a double asterisk. These women were delivered of 10,094 babies, 5035 in the electronic fetal heart monitoring group and 5058 in the intermittent auscultation group. Analyses based on these babies are marked with two daggers.

Comparability of allocated groups. Table I shows

Table II. Actual methods of intrapartum assessment††

	Allocated method							
Electronic fetal monitoring		Internittent auscultation						
Reason for nonuse	No.	%	Reaso z for nonuse	No.	%			
Delivered too rapidly	527	10.5	. Electronic fetal monitoring indicated					
Refused electronic fetal monitoring	331	6.6	Prolonged laLor	68	1.3			
Machine failure	37	0.7	Meconium	10	0.2			
No machine available	19	0.4	Fetal heart ree abnormality	21	0.4			
Other	.56	1.1	Other	20	0.4			
Allocated method used throughout labor	4066	80.7		4941	97.7			

Table III. Indication for fetal scalp blood sampling†

,	Electronic fet	Electronic fetal moritoring		Intermittent auscultation	
Indication for sampling	No.	%	No.	. %	
Fetal heart rate tracing abnormality	169	2.6	6	0.1	
Auscultated fetal heart rate abnormality	4.	. 0.1	43	0.6	
Labor >8 hr	77	1.2	139	2.1	
Meconium	24	0.4	28	0.4	
Other	14	0.2	. 16	0.2	
Total	286	4.4	232	3.5	

 $p < 0.025 (\chi^2 = 5.85, 1 df)$.

that randomization achieved comparability between the two groups in a number of important respects.

Compliance with allocated policy

Methods of fetal heart rate monitoring actually used during labor. The vast majority (97.7%) of women allocated to intermittent auscultation were monitored in this way throughout labor (Table II). Of those allocated to electronic monitoring, delivery was too rapid for the method to be set up in 10%. A further 6% refused the technique, most commonly because they wished to be completely mobile during labor or because they had previously been delivered in the National Maternity Hospital and associated electronic fetal heart monitoring with problems during labor. A small proportion could not be monitored electronically for technical or other reasons. In all, however, more than 80% of those allocated to electronic monitoring were monitored in this way.

Tracing quality and interpretation of electronic fetal heart monitoring. When reviewed following delivery, 11% of tracings made during the first stage of labor were unclassifiable because the tracing was of poor quality (5%), because the first-stage tracing lasted less than 10 minutes (3%), or because the tracing was mislaid (3%). A further 6% were judged to have had fetal heart rate patterns that would require clinical action if they did

not resolve spontaneously or did not respond to conservative measures (Details of the incidence of these "action patterns" will be published elsewhere). Of these action patterns, 31% did resolve spontaneously, 10% responded to conservative measures alone, 48% were considered to have prompted appropriately timed clinical action (usually a fetal blood sample), and 4% prompted appropriate but delayed action. In 13%, however, it was judged that there had been no appropriate clinical reaction.

Of mose women in the electronic fetal heart monitoring group for whom the fetal heart was monitored electronically during the second stage of labor, 39% had tracings that were unclassifiable, most commonly because of "poor quality" (18%) or a second stage of <10 minutes' duration (14%). A further 14% had tracing pæterns that were considered to "require clinical action 'Of these, 68% resolved spontaneously (usually as a result of celivery), 2% responded to conservative measures, 19% were considered to have had appropriate action (usually delivery with forceps), and 1% had appropriate but delayed action. In 9% it was judged that no appropriate clinical action had been taken.

Quarity and interpretation of intermittent auscultation. In the incermittent auscultation group the rate of noncomplance with the fetal heart rate monitoring pro-

Table IV. Scalp pH values in the "acidotic" and "preacidotic" range by indication: numbers and rates per 100 fetal blood samples taken

		pH <	<7.20			pH 7.2	20-7.25	
	Electro mons	nic fetal itoring	1	nittent ltation	Electro mont	nic fetal itoring		mittent iltation
Indication	No.	%	No.	%	No.	%	No.	%
Fetal heart rate abnormality	26	15.6	8	16.3	22	12.9	3	6.1
Labor >8 hr	2	2.6	7	5.0	4	5.2	17	12.2
Meconium	1	4.2	1	3.6	1	4.2	2	7.1
Other	0	0.0	0	0.0	0	0.0	1	6.3
Total	29	10.1	16	6.7	27	9.4	23	9.9

Table V. Randomization-delivery interval and use of oxytocin and analgesia**

		nic fetal toring	Intern auscul		
Parameter	No.	%	No.	%	Significance
Randomization-delivery interval >2 hr	2731	54.8	2943	58.9	p = 0.0001 ($\chi^2 = 15.3, 1 df$)
Oxytocin use	1122	22.5	1188	23.8	NS $(\chi^2 = 2.3, 1 \text{ df})$
Meperidine (maximum of 2 × 50 mg doses at interval of 4 hr)	2217	44.7	2388	48.0	$p < 0.001$ ($\chi^2 = 10.9, 1 \text{ df}$)
Epidural	143	2.9	167	3.3	NS ($\chi^2 = 1.4, 1 df$)

tocol was 4%. Of these, 80% were failures to record the fetal heart rate, particularly in the second stage of labor. In the remaining 20%, tachycardia or bradycardia was noted but no action was recorded as having been taken.

Labor, delivery, and puerperium. The overall rates of successful fetal scalp blood sampling were 4.4% in the electronic fetal heart monitoring group and 3.5% in the intermittent auscultation group ($\chi^2 = 5.85$, 1 df, p < 0.025). Table III contrasts the indications for fetal blood sampling in the two groups. More than three times as many fetal blood samples were obtained for fetal heart rate abnormalities in the electronic fetal heart monitoring group (2.7%) as in the intermittent auscultation group (0.7%, $\chi^2 = 69.8$, 1 df, p << 0.001). On the other hand, nearly twice as many fetal blood samples were taken in the intermittent auscultation group because labor had lasted for more than 8 hours (1.2% in the electronic fetal heart monitoring group, 2.1% in the intermittent auscultation group; $\chi^2 = 17.3$, 1 df, p < 0.001). Table IV shows the number of low scalp pH values in the two groups identified as a consequence of these contrasting policies. Overall, 4.4/ 1000 babies allocated to electronic fetal heart monitoring compared with 2.4/1000 allocated to intermittent auscultation were found to have a scalp blood pH of ≤7.20. The large difference between the two groups in the number of scalp blood pH values identified in the "acidotic" (<7.20) and "preacidotic" (7.21 to 7.25)

ranges because of fetal heart rate abnormalities was partially offset by more low pH measurements in samples in the intermittent auscultation group taken because labor had lasted for 8 hours (Table IV).

Mothers allocated to electronic fetal heart monitoring had shorter labors and, partly as a result of this, were less likely to have received meperidine (maximum of two doses of 50 mg) or an epidural analgesic (Table V). Those allocated to electronic fetal monitoring were only slightly more likely to have had a cesarean delivery (Table VI). This difference, however, was entirely due to more frequent use of cesarean delivery for a combination of a fetal heart rate abnormality and low fetal scalp blood pH. In 3.6/1000 mothers allocated to the electronic fetal heart monitoring policy compared with 1.1/1000 in the intermittent auscultation group, cesarean delivery was performed for this indication (χ^2 = 7.54, 1 df, p < 0.01). Cesarean delivery for other intrapartum emergencies such as prolapsed umbilical cord, abruptio placentae, or failure to progress in labor were equally common in the two groups. The marked difference in the rate of forceps delivery (Table VI) was almost totally explained by differences in the numbers of forceps deliveries prompted by abnormalities of the fetal heart rate.

The rates of puerperal pyrexia (defined as a single temperature ≥38° C) were 19.6/1000 in the electronic fetal heart monitoring group and 16.7/1000 in the in-

Table VI. Operative deliveries*

Type of delivery	,	Electronic fetal monitoring		Intermittent auscultation		
		No.	%	₹vo.	%	Significance
Cesarean deliveries—primary indication	;		•			_
Failure to progress in labor		84	1.3	88	1.3	
Fetal distress		25	0.4	10	0.2	
Other		49	0.7	46	0.7	
Total		158	2.4	144	2.2	NS
Forceps deliveries—primary indication						
Failure to advance		323	5.0	313	4.8	
Fetal distress	:	190	2.9	75	1.2	
Other		15	0.2	19	0.3	
Total	;	528	8.2	±07	6.3	p < 0.0001 ($\chi^2 = 16.9, 1 df$)

Table VII. Neonatal indices

		Electronic fe	tal monitoring	Intermittent	auscultation
Indication		No.	%	No.	%
Apgar score <3†		-			
At 1 min		57	0.9	62	1.0
At 5 min		10	0.2	5	0.1
Intubation††		58	1.2	54	1.1
Admission to SCN			•		
Overall†	•	547	8.4	543	8.3
For reasons which might have been affected by intrapartum care††	i	130	2.6	141	2.8
Umbilical cord venous pH‡		540		535	
<7.05		2	0.4	` 2	0.4
7.05-7.09	;	3	0.6	9	1.7
7.10-7.20	•	41	7.6	40	7.5
>7.20		494	91.4	484	90.4
Mean + SE		-	+ 0.003		+ 0.003

SCN = Special care nursery.

‡Based on 1075 consecutively delivered trial babies.

termittent auscultation group. The difference in these overall rates reflected a difference in the rates of pyrexia due to genital tract infections (8.5/1000 and ϵ .2/1000, respectively, $\chi^2 = 1.41$, 1 df, p = 0.24). This difference was not simply a reflection of the greater incidence of operative delivery in the electronic fetal heart monitoring group.

Neonatal outcome. The babies in the two groups had very similar chances of having an Apgar score of <4 at 1 and 5 minutes after delivery, of being intubated, and of being admitted to the special care nursery (Table VII).

Umbilical venous acid-base status was successfully measured in 1075 (95.5%) of 1125 consecutively de ivered trial babies. Although there was no difference in the mean pH of the two trial groups (Table VII) the proportion of babies with an umbilical venous \supset H <7.10 was somewhat lower in the electronic fetal heart monitoring group.

Neoratal trauma was rare overall, with a similar pattern in the two randomized groups (Table VIII). Serious trauma, however, was more common in the electronic fetal heart monitoring group (3.2/1000 compared with 2.4/1000 in the intermittent auscultation group). Neonatal infection rates (0.8%) were the same in the two groups. In particular, there were no cases of scalp abscess in either group.

There were 0.4% of babies in each group with congenital abnormalities considered to have caused death or abnormal neurological features. As prespecified in the protocol, these babies have been excluded from the data presented in Tables IX to XIV. The frequencies of the principal measures of adverse outcome in the first 10.094 babies are summarized in Table IX. The overall rate in the intermittent auscultation group (9.5/1000) was close to the 10/1000 expected (see above). There were more of these events among those cases allocated to intermittent auscultation ($\chi^2 = 2.77$, 1 df,

Table VIII. Neonatal trauma††

	Electron	c fetal monitoring	Intermittent auscultation	
Trauma	No.	per 1000	No.	per 1000
Subdural hemorrhage with death	3	0.6	1	0.2
Fractured clavicle	4	0.8	2	0.4
Motor deficit in upper arm	7	1.4	. 6	1.2
Facial nerve injury	2	0.4	3	0.6
Scalp laceration, abrasion, or bruising	13	2.6	15	3.0
Cephalhematoma	10	2.0	10	2.0
Facial bruising, suffusion, forceps marks, and conjunctival hemorrhage	11	2.2	11	2.2
Other bruising	21	4.2	18	3.6
Total	71	14.1	66	13.0
Relative risk		1.08		1.00
95% Confidence limit		0.76	-1.54	

p < 0.10). The numbers of deaths were identical, however, and the overall difference reflected differences in the frequency and pattern of abnormal neurological characteristics. In all three of the mutually exclusive categories of abnormal neurological characteristics in babies who went on to survive the neonatal period, there were more cases among those allocated to intermittent auscultation than among babies who had been allocated to electronic fetal heart monitoring. These differences were most striking for cases of seizures and cases of neurological abnormality persisting for a week or more. When all 13,084 babies in the trial are considered, there were significantly fewer neonatal seizures among those allocated to electronic monitoring (Table X). This difference was reflected both among those infants who survived the neonatal period and among those in whom seizures were followed by neonatal death. In 10 of the 12 cases in the electronic fetal heart monitoring group and 24 of the 27 cases in the intermittent auscultation group, seizures were first noted within 48 hours of birth. In four of the five "late" cases the underlying cause is unlikely to be related to events during labor (one case of meningitis in a baby of 28 weeks' gestation, two cases of complications of hyaline membrane disease, and one case of hypoglycemia); in the fifth, seizures were first noted at 56 hours of age.

The overall risk of intrapartum and neonatal death in normally formed infants (2.1/1000) was the same in the two groups, and the timing of death in relation to labor was similar (Table XI). Seven deaths in each group were primarily attributed to "asphyxial conditions developing during labor" (Table XII). In one further case in each group asphyxial conditions may have been contributory. Of the cases primarily related to asphyxial conditions, one case in the electronic fetal heart monitoring group and two cases in the intermittent auscultation group had a documented failure to follow the fetal heart rate management protocol (this applied to one stillbirth in each group); three cases in the electronic fetal heart monitoring group and four

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Table IX. Frequencies of prime measures of neonatal outcome in first 10094 trial babies††

Outcome	Electronic fetal monitoring	Intermittent auscultation
Death		
Intrapartum stillbirth	1	1
Neonatal death	6	6
Neurological abnormality in survivors	•	
Seizures '	9	16
Other simultaneous abnor- malities of tone and reflexes	4	5
Other abnormal neurolog- ical signs persisting for ≥1 wk	12	20
Total	32 (6.4/1000)	48 (9.5/1000)
Relative risk 95% Confidence limit	0.67 0.43-1.04	1.00
χ^2 (1 df, p < 0.10)	2.77	

cases in the intermittent auscultation group were apparent failures of the respective fetal heart rate monitoring policies (all neonatal deaths) and the remaining three electronic fetal heart monitoring cases and one intermittent auscultation case were associated with failures in aspects of the hospital's policy other than fetal heart rate monitoring (these included the other three intrapartum stillbirths). Of nine deaths that followed neonatal seizures, six were primarily ascribed to "asphyxial conditions developing during labor," two in the electronic fetal heart monitoring group and four in the intermittent auscultation group.

The three deaths ascribed to trauma in the electronic fetal heart monitoring group were all due to tentorial tears associated with forceps delivery prompted primarily by failure to advance in the second stage of labor. The traumatic death in the intermittent auscultation group was due to a tentorial tear in a second twin delivered vaginally by the breech. In each group there

Table X. Neonatal seizures†

Sequela of seizures	Electronic fetal monitoring	Intermittent auscultation	Significance
Neonatal death Survival	3	6 21	0.05
		. 21	$p < 0.05 (\chi^2 = 4.00, 1 df)$
Total	. 12	27	p = 0.025 ($\chi^2 = 4.99, 1 df$)
Relative risk 95% Confidence limit	0.45 0.22-0.91	1.0	, , ,

Table XI. Stillbirths and neonatal deaths†

	Electronic fetal monitoring	Intermittene auscultatio2
Intrapartum stillbirths	. 3	2
Neonatal deaths‡	11	12
Total	14	14

[‡]These include the nine deaths following neonatal seizures.

were two neonatal deaths of babies who were discharged from the hospital apparently well. Two of these died because of fractures of the skull resulting from trauma at home, one was due to the sudden infant death syndrome, and the fourth was caused by necrotizing enterocolitis.

In summary, when the major adverse outcomes in the two groups of neonates as a whole are compared, the outstanding difference relates to the frequency of abnormal neonatal neurological characteristics among babies who survived.

Follow-up examination at 1 year of age has been successfully accomplished in 42 of the 43 babies who survived seizures and other simultaneous abnormalities of tone and reflexes in the neonatal period. At this examination, three babies in each trial group, all of whom had experienced neonatal seizures, were judged to have major neurological disabilities, including cerebral palsy.

Stratified analyses. A total of 22.5% of mothers met one or more of the predefined criteria for "high-risk" cases. Compared with those who met none of these criteria, these women were 2.7 times more likely to have had a cesarean delivery. Furthermore, their babies were more than three times more likely to have had an Apgar score of <4 at 1 minute, to be admitted to the special care nursery, or to suffer a stillbirth or neonatal death. Within the risk groups, however, there was no evidence suggesting a differential effect of the two policies on these measures of outcome. Table XIII shows the distribution of the 39 cases of seizures in the two risk groups. Overall, the frequency of seizure cases in the high-risk group (4.3/1000) was greater than that ir the

low-risk group (2.6/1000). Despite this, the effect in cases of seizures prevented was apparently greater in the low-risk group (1.8 seizure cases per 1000 high-risk babies electronically monitored compared with 2.4 seizure cases per 1000 low-risk babies electronically monitored) In contrast, the risk of seizures followed by survival was almost identical in the two risk groups (2.3/1000), as was the apparent effect in seizure cases prevented by electronic fetal heart monitoring (1.9 seizure cases per 1000 babies electronically monitored in the high-risk stratum and 1.8 seizure cases per 1000 babies electronically monitored in the low-risk stratum).

The overall difference in seizure rates reflected the experience of the 75% of babies for whom the interval between random treatment allocation and delivery was more than 1 hour. Scalp blood sampling and forceps delivery were more common in the electronic fetal heart monitoring group among babies who were delivered within 1 hour of entry. Despite this, there were equal numbers of babies who survived seizures (two) in each group who were born during this time period.

This analysis suggested a relationship between the effect of electronic fetal heart monitoring and the duration of labor. For this reason an additional stratified analysis was performed to assess the effect of the two policies in relatively short and relatively long labors (Table XIV). The cutoff point (5 hours) for defining these two groups was chosen prior to the inspection of the data and was intended to generate strata with similar numbers of seizure cases. The 15% of labors that lasted more than 5 hours included 62% of the neonatal seizure cases and demonstrated all the protective effects of electronic fetal heart monitoring. In this subgroup there was a nearly fourfold difference in the rate of neonatal seizures associated with the two trial policies. Oxytozin was more commonly used in longer labors. Further analysis (Table XIV) (which was not prespecified in the protocol and is also based on very small numbers) suggested that this difference in seizure rates in labors lasting more than 5 hours existed whether or not oxytocin had been used to induce or accelerate labor. In shorter labors, however, a lower seizure rate

Table XII. Pathologic subgroups of stillbirths and neonatal deaths†

Primary cause of stillbirths and neonatal deaths	Electronic fetal monitoring	Intermittent auscultation
Asphyxial conditions developing in labor	77	7
Asphyxial conditions developing in labor Conditions associated with immaturity	I	4 (1)
Birth trauma	3 (1)	- 1
Other	3	2
Total	14	14

Figures in parentheses refer to the numbers of cases in which asphyxial conditions developing during labor may have been contributing factors but were not considered to be the primary cause of death.

Table XIII. Distribution of 39 cases of neonatal seizures by risk group and allocated group†

	Electronic fetal monitoring			Intermittent auscultation		ifference
	No.	per 1000	No.	per 1000	No.	per i 000
Pregnancy risk factor present	1492		1539			
Seizures followed by neonatal death	3	2.0	3	1.9	0	+0.1
Seizures followed by survival	2	1.3	5	3.2	3 .	-1.9
Total	5	3.4	8	5.2	3	-1.8
Pregnancy risk factor not present	5038		5015			
Seizures followed by neonatal death	0	0.0	3	0.6	3	-0.6
Seizures followed by survival	7	1.4	16	3.2	9	-1.8
Total	7	1.4	19	3.8	12	-2.4

Table XIV. Distribution of 39 cases of neonatal seizures by duration of labor and use of oxytocin in labor†

	Electronic fetal monitoring		Intermitte	nt auscultation
	No.	per 1000	No.	per 1000
Labor <5 hr				
Oxytocin used in labor	0	0.0	4	11.7
Oxytocin not used in labor	7	1.7	4	1.0
Total	7	1.6	8	1.8
Labor >5 hr			_	
Oxytocin used in labor	4	3.5	15	12.3
Oxytocin not used in labor	1	1.1	4	4.0
Total	5	2.4	19	8.5

in the electronic fetal heart monitoring group was apparent only in cases in which oxytocin had been used during labor.

Comment

The National Maternity Hospital in Dublin has a larger number of births than any other maternity unit in the British Isles. There is a uniform approach to the conduct of labor which has been described in detail by O'Driscoll and Meagher.11 In particular, there is a nurse-midwife with each woman, progress is assessed at least every 2 hours, and oxytocin is used to accelerate slow labor in primigravid women at an early stage. (In the study population, 23% of women received oxytocin.) The amniotic fluid is inspected early in labor, and in the 5% of cases in which either the fluid is significantly stained with meconium or no fluid is seen the fetus is deemed to be at special risk. These cases were not eligible for this trial. All other cases of at least 28 weeks' gestation, regardless of risk status in other respects, were eligible for entry once a diagnosis of labor had been made.

Prior to the trial, these eligible cases with normal amniotic fluid were supervised with intermittent auscultation and fetal scalp blood pH estimation where indicated; thus the hospital had extensive experience with this method of fetal monitoring. It also had considerable experience of electronic fetal heart monitoring because this technique had been used routinel—in about 500 cases annually in which the fetus was considered to be at special risk. Furthermore, the setior resident obstetricians have all received training out=de the Republic of Ireland in hospitals in which electronic fetal heart monitoring is used more or less routinely. Fetal scalp blood sampling is commonly used in the hospital, not only as an adjunct to fetal heart monitoring but also in cases in which there was either abeent amniotic fluid or significant meconium staining of the fluid and in labors of more than 8 hours' duration.

· It is important to stress again that the design of his trial was "pragmatic" (as opposed to "explanatory", to use the terminology of Schwartz et al.22 This implies the comparison of subjects who have been randomly allocated to one of two clinical policies of intrapartim fetal heart monitoring, as these would be used in everyday clinical practice in this hospital. This experime atal design takes into account the reality that sometimes it will not be either possible or appropriate to apply throughout labor the method of fetal monitoring selected, despite an intention to do this at the time of entry to the trial. The design does, of course, dep-nd on the large majority of cases being monitored with the allocated method, as was achieved in this trial. According to this pragmatic design the extra time taken to set up electronic fetal heart monitoring compared with the time for intermittent auscultation is considered to be an integral part of the electronic fetal heart monitoring policy. Some women (10.5% in this study) are delive ed too quickly for this to be successfully accomplished. Fetal surveillance might be suboptimal while electronic fetal heart monitoring is being organized and before a satisfactory tracing is available. There was no evidence from the stratified analysis based on time interval between trial entry and delivery to suggest that this occurred. The number of adverse outcomes in this a 1alysis was small, but the differential effects of the wo policies did appear to be restricted to those who were in the trial sufficiently long to be monitored with the method to which they had been allocated.

Because women in the intermittent auscultation group were more likely to be mobile during labor it had been predicted that their labors would be shor er; in fact their labors were longer (Table V). Comparable data from other randomized trials^{6,8} (S. Neldam, Eersonal communication) shows no clear pattern. One gossible mechanism for such an effect in this study is the extra vaginal manipulation with associated release of prostaglandin in the electronic fetal heart monitoring group. Women in the electronic fetal heart monitoring group were also more likely to be delivered by force ps, but this difference did not explain their shorter labors.

The less frequent use of opiate and epidural analgesics in the electronic fetal heart monitoring gr-up (Table V) was also unexpected. Some of this difference is explained by the shorter duration of labor. When this factor is taken into account the difference in use of mepericline (a maximum of 2 doses of 50 mg) just fails to reach statistical significance at the 5% level. Comparable data are available in five of the other six randomized controlled trials. No clear pattern emerges from these; in three of these studies more intramuscular analgesic was given to the intermittent auscultation groups and in two the rates were higher in the electronic fetal heart monitoring groups.

The inding of a low cesarean delivery rate and little difference between the rates associated with the two policies is in contrast with the results of the other randomized controlled trials. We believe that the pattern in this trial reflects first, the use of fetal scalp blood pH estimation in both trial groups; second, the strict audit of cesarean deliveries at the National Maternity Hospital; and third, the infrequent use of epidural anesthesia, a form of pain relief that is known to predispose to fetal heart rate abnormalities.²³

Electronic fetal heart monitoring identified nearly twice as many fetuses with low scalp blood pH (Table IV). The threefold difference in the number identified as a consequence of an abnormal heart rate was partially offset by a difference in the number identified when the duration of labor exceeded 8 hours (Table IV). Established hospital practice (which applied to the intermittent auscultation group) of sampling fetal blood after 8 hours was modified in the electronic fetal heart monitoring group; if the fetal heart rate tracing was normal and labor was progressing satisfactorily, fetal blood sampling was omitted. For this reason, twice as many blood samples were taken for this indication in the intermittent auscultation group (Table III). To some extent this diluted the differences between the two monitoring policies (Table IV). Nevertheless, twice as many cesarean deliveries were prompted by a low scalp pH in the electronic fetal heart monitoring group as compared with the intermittent auscultation group and this entirely explained the difference in the overall cesarean delivery rates (Table VI). In contrast, the small number of forceps deliveries prompted by low scalp pH was similar in the two groups and the marked difference in the overall rate was almost totally explained by differences in the numbers of forceps deliveries performed for abnormalities of the fetal heart rate alone (Table VI).

The small difference (3/1000) in the rates of puerperal pyrexia (defined as one temperature reading of 38° C cr more) was explained by the differences in the rates of genital tract infection. This finding was reinforced by an in-depth study in 2716 of the women in the trial. All cases in which the temperature reached 37.2° C at least 24 hours after delivery were screened

for infection. Genital tract infection was three times more common in the electronic fetal heart monitoring group ($\chi^2 = 4.01$, 1 df, p < 0.05) but rates of other infections were similar in the two groups. These findings are consistent with the findings in other randomized controlled trials3-5 and also some observational studies,1 although intrauterine catheters were not used in the present study.

Studies using observational data1 have also suggested that babies are at increased risk of infection when internal monitoring is used. This was not confirmed by this study or by the pattern of results in the six other randomized controlled trials. In more than 5000 cases in this trial where a scalp electrode was applied not a single scalp abscess was found.

Neonatal trauma rates were similar in the two groups. Most major trauma was sustained by babies delivered vaginally either with forceps or by the breech. The exceptions were cases of Erb's palsy in large babies delivered spontaneously. Babies allocated to electronic fetal heart monitoring were 33% more likely to be delivered by forceps than those managed with intermittent auscultation. All three deaths ascribed to traumatic intracranial hemorrhage following forceps delivery were in the electronic fetal heart monitoring group. This may be a chance difference, but it is consistent with recent reports suggesting that the incidence of fetal trauma is directly related to the frequency of operative vaginal delivery.24.25 The few cases of scalp trauma were associated with fetal scalp blood sampling. There was little evidence that use of the scalp electrode per se caused trauma; this contrasts with the findings of observational studies using other types of electrodes.1

There was no difference between the two groups of babies at birth as judged by Apgar scores, need for endotracheal intubation, and admission rates to the special care nursery (Table VII). In the subsample for which umbilical cord venous pH was successfully measured, the proportion of values <7.10 was lower in the electronic fetal heart monitoring group, but this observation is based on small numbers and does not reach statistical significance.

There were equal numbers of deaths in the two groups and these occurred at similar times in relation to labor. It is notable that the mortality rate (stillbirths and neonatal deaths) of babies included in the trial (2.1/ 1000) is substantially lower than the rate in babies ineligible for the trial because of meconium or no visible amniotic fluid (11.4/1000) and also lower than the rate experienced by those who were delivered too rapidly to enter the trial (3.5/1000).

Poor compliance with the protocol was associated with one death in each group. Electronic fetal heart monitoring tracings that "required clinical action" did prompt appropriate action in most cases. In the minority of cases (0.8% in the first stage and 1.3% in the second) in which appropriate action was not taken, there was one case of neonatal seizures and two cases of persistent abnormal neonatal neurological features. Compliance with the intermittent auscultation policy was more difficult to assess. The case-note review revealed that in 0.8% of cases an auscultated fetal heart rate abnormality was recorded but apparently not acted on.

There were significantly fewer cases of neonatal seizures among those allocated to electronic fetal heart monitoring. This finding is consistent with the results of the pooled analysis conducted by Chalmers9 on data derived from the other randomized trials which compared electronic fetal heart monitoring plus scalp blood sampling with intermittent auscultation. The difference in seizure rates in this trial was reflected among both those infants who survived the neonatal period and those in whom seizures were followed by neonatal death (Table X). Overall, the electronic fetal heart monitoring policy was associated with a 55% reduction in the frequency of neonatal seizure cases. This estimate is, however, compatible with a real reduction of as little as 9% or as great as 78%. Looked at another way, the result suggests that to prevent one case of neonatal seizures it is necessary to monitor continuously 433 fetuses, but this figure may be as low as 240 or as high as 2167 fetuses monitored per case of seizures prevented.

Babies with other categories of abnormal neonatal neurological features were also less common in the electronic fetal heart monitoring group (Table IX). The main hypothesis tested on the first 10,094 babies delivered in the trial was that the number of these cases combined with the number of deaths and seizure cases would be reduced by more intensive fetal monitoring (see section on hypotheses and sample size). The observed reduction of a third just failed to reach conventional levels of statistical significance (Table IX). Among these 10,094 babies, the numbers of deaths were identical, however, and the observed difference in the number of cases with abnormal neurological features (25 in the electronic fetal heart monitoring group compared with 41 in the intermittent auscultation group) is unlikely to be due to chance ($\chi^2 = 3.4$, 1 df, p = 0.07).

Despite observations suggesting that some "highrisk" fetuses have a relatively low tolerance for intrapartum asphyxia,26 we were unable to find any reflection of such a phenomenon in the prespecified stratified analysis based on risk status.

The differential effect of the two policies on neonatal seizure cases did appear to be related to duration of labor. In a secondary analysis, the difference in the seizure rate was found to be limited to the 15% of babies delivered after a labor of more than 5 hours (Table XIV). Within this group there was a nearly fourfold difference and this appeared to be independent of oxytocin use during labor (Table XIV). Unlike the other results presented, this finding is not based on an analysis which had been prespecified in the trial design. Furthermore, it is based on small numbers. Nevertheless, it is plausible that electronic fetal heart monitoring could have this effect. When labor is prolonged, abnormal fetal heart rate patterns (P. Steer, unpublished observations), low fetal scalp pH,²⁵ and neonatal seizures (P. Mincham et al., unpublished observations) are all more common. This hypothesis, however, should be tested formally in any future randomized comparisons of alternative methods of intrapartum fetal monitoring.

What is the longer-term significance of the differences we have found in the incidence of neurologically abnormal neonates in the two trial groups? Although the total number of deaths in the two trial groups was similar, our study confirmed the pattern of death following neonatal seizures suggested by Dennis and Chalmers' review of the world literature: Nine of 39 babies (23%) who had seizures died in the neonatal period, and in six of these nine cases, death was ascribed at necropsy to asphyxial conditions developing during labor.

Abnormal neurological signs in the neonatal period are also good predictors of longer-term status in survivors. 10, 27 In particular, neonatal seizures are strongly associated with childhood disability, particularly cerebral palsy. Examination of children in our study 1 year after they experienced neonatal seizures or other simultaneous abnormalities of tone and reflexes has so far revealed six children with severe disabilities. This is a lower proportion than would be expected from Dennis and Chalmers' review and this is certainly in part a reflection of the short period of follow-up. Despite the marked difference in the total numbers of seizure cases in the two trial groups, equal numbers of babies in each group were found to have severe disabilities at 1 year of age. The nature and extent of any relationship that exists between "intrapartum asphyxia" and long-term disability remain controversial28, 29 and are discussed elsewhere.12

We plan to review at 4 years of age all babies who exhibited abnormal neurological signs in the neonatal period. These reexaminations will include psychological and neurological assessments as well as tests of hearing, sight, and speech when indicated. Cases of cerebral palsy among babies who did not have abnormal neurological signs in the neonatal period and who therefore will not have been followed up systematically will be identified at the time of referral to specialist clinics.

The implications of the findings presented in this report can be considered in the light of what they contribute to the development of both theory and practice.

We be leve that the results of this large human experiment provide important evidence concerning the way in which obstetrically preventable intrapartum fetal asphyxia manifests itself during the neonatal period. Our results suggest that such asphyxia is reflected in earlyonset neonatal seizures (and other abnormal neurological signs, similar to the premonitory signs of seizures) but nct in decressed Appar scores. Indeed, the frequence of depressed Apgar scores at all levels was so similar in these two large, randomized cohorts exposed to different degrees of fetal asphyxia that the validity of the Apgar score as an indicator of preventable intraparium asphyxia is seriously called into question. Others have already drawn attention to the poor correlation between Apgar scores and umbilical cord blood gas values.12 The extent to which the latter reflect preventable intrapartum fetal asphyxia also remains unclear. Although the randomized cohorts reported here are larger than any others reported in the literature, the suggestion from these data that levels of cord venous pH <7.10 might reflect preventable fetal asphyxia does not reach conventional levels of statistical significance. Larger experiments are required to assess the validity of cord blood gas measurements both as indicators of obstetrically preventable intrapartum fetal asphyxia and as predictors of long-term status in childhood.

The implications of our findings for obstetric practice must recessarily depend on judgments concerning the significance of preventing intrapartum fetal asphyxia and thereby reducing abnormal neurological signs during the neonatal period. These abnormalities are often disturbing for parents and staff alike. Whatever their longer-term implications may turn out to be, some people will undoubtedly feel that it is important to prevent them by more intensive intrapartum fetal monitoring in all abors. Others who wish to use electronic fetal heart monitoring and scalp sampling more selectively may choose to base their practice on the results of the stratified analysis which suggests that the benefits of the more intensive method of fetal monitoring are restricted to relatively long labors. Although there are substantial reasons for thinking that such a judgment would be rational, we must reiterate that this analysis was not prespecified in the trial protocol and that it cannot rule out the possibility of a differential effect of the two policies in shorter labors. Some people will wish to await the results of further follow-up assessments of babies who were neurologically abnormal as neonates before contemplating any extension of a far more restricted use of more intensive methods of intrapartum fetal monitoring. Last, there will be others who will conclude that our results are not easily generalizable because they may simply reflect the specific context in which the trial was conducted. The implications of our results for them are that they should mount further trials in other settings to test their hypotheses.

We would end by stressing that in selecting from these and other policy options it will always remain important to take into account both the wishes of individual women in labor30 and wider issues such as alternative uses of the additional resources required for more extensive use of intensive intrapartum fetal monitoring.

A project of this size requires the collaboration of large numbers of people and we are grateful to all those who have helped us. In particular, we thank Noemi Mackey and Marie-Therese Joy for so efficiently performing the day-to-day data collection; Valerie Greig and her nursing colleagues in the delivery suite and on the postnatal wards; Colm O'Herlihy, Patricia Crowley, Michael Foley, Michael Turner, and all members of the resident medical staff; Niall O'Brien for initiating and assuming overall responsibility for follow-up studies and Margaret Moore for help in organizing these; Margaret Lett and Della O'Donnell and their colleagues in the Medical Records Department; Loretta O'Connor at the Computer Centre, University College, Dublin; the staff of the Central Statistical Office, Dublin; Michael Gillmer, David Baum, and Klim McPherson for their assessment of the interim results and statistical advice; and Al Haverkamp, Miriam Orleans, Richard Beard, Ian Cooke, and Ted Quilligan for advice on the trial design.

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Maternal serum α -fetoprotein screening: The effect of participation on anxiety and attitude toward pregnancy in women with normal results

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The purpose of this study was to assess the psychological impact of maternal serum α -fetoprotein screening on pregnant women with normal results and their male partners. Assessments of anxiety (as measured by the state-trait anxiety inventory) and attitudes toward pregnancy (as measured by the maternal attitude to pregnancy instrument) were conducted sequentially beginning at 16 weeks' gestation in women participating in the maternal serum α -fetoprotein screening with normal results and in women without access to screening. Screened subjects exhibited similar or lower lenels of anxiety at each point in time as compared to unscreened subjects. In addition, they exhibited a similar or more positive attitude toward pregnancy. No differences in anxiety were observed between male partners in the two groups. Participation in screening appeared to have no adverse psychological effect on the subjects studied. (AM J OBSTET GYNECOL 1985;152:540-3.)

Key words: α-Fetoprotein, pregnancy, screening

During the past decade, it has been repeatedly demonstrated that measurement of maternal serum α-fetoprotein is an effective screening tool for the identification of the majority of fetal neural tube defects. Although many of the original studies were conducted in the United Kingdom,1 several large pilot studies in the United States have demonstrated the effectiveness and feasibility of maternal serum α-fetoprotein screening in the United States.2-4 These developments have generated considerable controversy regarding the advisability of including maternal serum α-fetoprotein screening in routine obstetric care.5.5 Much concern has centered on the potential adverse psychological impact of screening on the pregnant woman. We have recently demonstrated that women with maternal serum \alpha-fetoprotein elevations experience significant anxiety during the course of subsequent testing but, if further testing yields normal findings, do not exhibit heightened anxiety or altered attitudes toward pregnancy during the remainder of the pregnancy.7 It could still be argued, however, that the mere exposure to maternal serum αfetoprotein screening, even among women with normal test results, could lead to heightened anxiety or altered

attitudes that would not be apparent without examining these variables in women not exposed to such a screening program.

The purpose of the present study was to compare anxiety and attitudes toward pregnancy in women undergoing maternal serum α -fetoprotein screening with normal results and in those without access to a screening program.

Material and methods

Screened subjects in the study were participants in a screening program for neural tube defects in North Carolina and served as controls in a study of anxiety and attitudes toward pregnancy among patients with maternal serum α-fetoprotein elevations.⁷ Details of the screening program in general8 and the methods of selection and recruitment of subjects⁷ have been previously described. The instruments used in the study were the state-trait anxiety inventory9 and the maternal attitude to pregnancy instrument.10 The state-trait anxiety in entory consists of a 20-item "A-state" scale [designed to assess the level of relatively transient situationrelatec ("state") stress perceived in a particular situation] and a 20-item "A-trait" scale (designed to measure the relatively stable long-term "resting level" of anxiety in the individual). The state-trait anxiety inventory has been subjected to intensive validity and reliability testing and is suitable for repeated administration. The maternal attitude to pregnancy instrument consists of 48 items with which the respondent must strongly agree, disagree, or strongly disagree. It is designed to assess attitudes toward pregnancy along several dimen-

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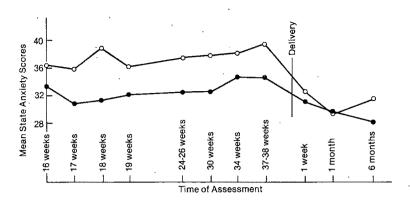


Fig. 1. State-trait anxiety inventory A-state scores in screened and unscreened subjects. •, Screened; o, unscreened.

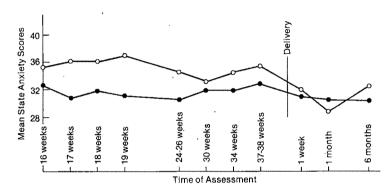


Fig. 2. State-trait anxiety inventory A-state scores in male partners of screened and unscreened subjects. •, Screened; o, unscreened.

Table I. Characteristics of study groups

	Screened	Unscreened	Difference*
No. of subjects	145	44	
Percent white	100	100	NS
Percent primigravid	59	36	NS
Mean age	29	28	NS
Percent planned pregnancies	77	69	NS
Hollingshead scale (% in each class)	•		p < 0.001
I	15	7.	*
II	34	16	
III	30	25	
IV	21	50	
V	0	2	

^{*}As determined by χ^2 analysis.

sions, including fearfulness, attitudes toward child, attitudes toward doctor, concern over child's normalcy, and general well-being. It has not been as extensively evaluated as the state-trait anxiety inventory with regard to its suitability for repeated administration. The schedule and method of questionnaire administration were as previously described.7

Unscreened subjects were recruited from a neighboring county in North Carolina not served by the screening program for neural tube defects. All neighboring counties without access to screening are rela-

tively sparsely populated, so that fewer unscreened subjects were available for study. During a 1-year period, cooperating physicians approached patients at their first prenatal visit and asked if they would be willing to discuss with project staff possible participation in a study designed to assess parents' feelings during pregnancy. Those who expressed a willingness to be contacted were subsequently called by the study coordinator and a home visit was scheduled at 16 weeks' gestation. At this time, the sequence of assessments was explained, and subjects who agreed to participate were

[†]Distribution among Hollingshead classes of screened versus unscreened subjects.

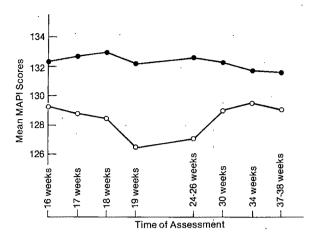


Fig. 3. Maternal attitude to pregnancy inventory scores in screened and unscreened subjects. •, Screened; o, unscreened.

given the first set of questionnaires. Subsequent assessments were conducted on the same schedule and in the same fashion as for screened subjects.

Results

The characteristics of the screened and unscreened groups are shown in Table I. Significant differences were observed between groups only for socioeconomic status as determined by Hollingshead's two-factor index of social position. Screened subjects tended to be better educated, and larger numbers were professionals; therefore, more controls than experimental subjects were found in the higher socioeconomic classes (I and II).

The state-trait anxiety inventory A-trait and A-state scores and the maternal attitude to pregnancy instrument scores were analyzed by multivariate analysis of variance, controlling for social class, gravidity, and planned versus unplanned pregnancy. Screened and unscreened groups were compared at each point in time

There was no significant difference in trait anxiety between screened and unscreened subjects (male or female). Results of the sequential assessments of state anxiety in female subjects are illustrated in Fig. 1. A significant difference between groups is observed only at the 18-week assessment, at which time the unscreened subjects were significantly more anxious than the screened subjects (p < 0.001). Both groups exhibited a gradual rise in anxiety as the delivery date approached, with a significant fall by 1 month following delivery.

The course of anxiety in male subjects is illustrated in Fig. 2. No significant differences were observed between partners of screened and unscreened subjects.

The sequential assessments of maternal attitudes toward pregnancy are illustrated in Fig. 3. Although scores of unscreened subjects were generally lower than those of screened subjects throughout pregnancy, reflecting a more negative attitude toward pregnancy, the difference was significant only at the time of the 24- to 26-week assessment (p < 0.02).

Comment

The effect of the presence of a screening program for neural tube defects in the community on patient anxiety has been studied in Sweden by Berne-Fromell and Kjessler.12 They found lower levels of anxiety in middle and late pregnancy in women living in an area with a maternal α-fetoprotein screening program than in wornen living in an area without access to screening. In adcition, Berne-Fromell et al.18 documented similar degrees of anxiety in patients who accepted screening and had normal results and in those who refused screening. Our results are similar to theirs in that we have cemonstrated that participation in screening has no adverse effect on the general level of anxiety observed among patients with normal maternal α-fetoprotein screening results. In fact, the one significant difference observed between screened and unscreened subjects reflected a higher level of anxiety in unscreened subjects at 18 weeks' gestation. Since this observation was not accompanied by the finding of significantly higher anxiety at any of the other assessments, it may have been a spurious observation. In any case, our data and those of Berne-Fromell and Kjessler12 would seem to lay to rest the argument that the presence of a screening program in a community may raise the general level of anxiety among pregnant women.

Our data also indicate that participation in screening has little or no effect on maternal attitudes toward pregnancy. If there is any effect at all, it would appear to be a beneficial one. The only significant difference in attitudes between groups occurred at 24 to 26 weeks' gestation, at which time the unscreened subjects exhibited a more negative attitude toward pregnancy. Since this difference occurred only at a single point in time, it is again difficult to know if it is of real significance.

Maternal α -fetoprotein screening is an effective tool in the prenatal detection of neural tube defects. We have previously demonstrated that the anxiety experienced by patients with maternal serum α -fetoprotein screening elevations is transient and is totally alleviated by normal findings during the course of subsequent testing. We demonstrate here that participation in maternal serum α -fetoprotein screening has no significant effect on anxiety or attitudes toward pregnancy in patients with normal maternal serum α -fetoprotein results. Given the appropriate setting, these findings lend further support to our belief that maternal serum α -fetoprotein screening should be a routine part of obstetric care.

We acknowledge the support and cooperation of the participating physicians in Forsyth, Guilford, and Davidson counties without whom this project could not have been completed. Dr. C. Drew Edwards was instrumental in study design. Mr. Bruce Kardon provided valuable statistical assistance.

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Fetal echocardiography

V. M-mode measurements of the aortic root and aortic valve in secondand third-trimester normal human fetuses

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The aortic root dimension and aortic valve excursion of 43 normal fetuses were recorded with M-mode echocardiography and the measured dimensions correlated with noncardiac measurements (biparietal diameter, head circumference, abdominal circumference, and femur length) and cardiac measurements (diastolic biventricular inner dimension, diastolic left ventricular internal dimension, and mitral valve excursion). The correlation coefficients for the aortic root dimension ranged between 0.87 and 0.95, while those for the aortic valve excursion ranged between 0.78 and 0.87. Regression analysis demonstrated that the best fit of the data was a linear model from which the 5% and 95% confidence limits were computed for individual predictions of aortic root dimension and aortic valve excursion from noncardiac and cardiac measurements. (AM J OBSTET GYNECOL 1985;152:543-50.)

Key words: Aorta, ultrasound, fetus, echocardiography, M-mode

With the introduction of diagnostic ultrasound, it is now possible to image the fetus and evaluate a number of organ systems. During the past few years reports

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have been published in which investigators have described normal anatomy of the fetal heart using real-time and M-mode ultrasound. ¹⁻³ More recently, data have been published in which M-mode cardiac chamber dimensions, ⁴⁻⁶ ventricular and septal wall thickness, ⁴⁻⁵ and atrioventricular valve excursion have been reported for the developing fetus. Although the above information is important for evaluation of the ventricular inflow tracts, it is also necessary to be able to assess the outflow tracts as well. Because of their position within the chest, the aortic root and valve are easy to

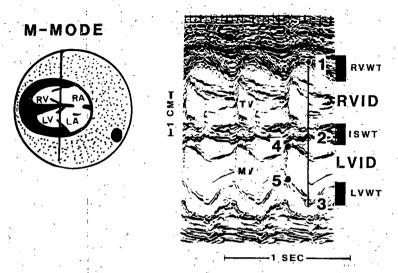


Fig. 1. Fetal ventricular measurements. The M-mode echocardiogram was recorded by directing the cursor perpendicular to the interventricular septum at the level of the atrioventricular valves. From the tracing the following measurements were obtained: diastolic biventricular inner dimension (1 to 3), left ventricular internal dimension (2 to 3), and mirral valve opening excursion (4 to 5). RV = Right ventricle; LV = left ventricle; RA = right atrium; LA = left atrium; TV = tricuspid valve; TV = mitral valve; $TV = \text{mitra$

image and an M-mode recording is easily obtained In addition, there are a number of congenital malformations involving the aortic root and/or valve that have major implications for the neonate once birth occurs. For these reasons, this article will report the results from an ongoing study in which M-mode dimensions of the aortic root and valve were correlated with ultrasound-determined fetal growth and ventricular parameters from normal fetuses between 18 and 41 weeks of gestation.

Material and methods

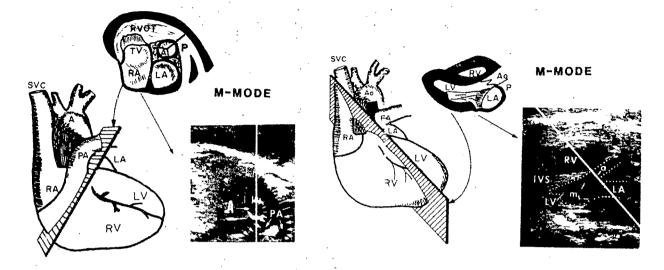
Forty-three normal fetuses between 18 and 41 weeks of gestation were included in the study. There was no history of maternal diseases that would predispose to abnormal fetal growth or development. Examination of the fetal head, trunk, and extremities was accomplished with a linear array system (Siemens 2380 or General Electric RT 3000), and the following noncardiac growth parameters were measured: biparietal diameter,7 head circumference,8 abdominal circumference,9 and femur length.10 Real-time evaluation of the following cardiac structures was accomplished as previously described: right and left atrial and ventricular chambers, interatrial and interventricular septa, fcramen ovale, mitral and tricuspid valves, aortic and pulmonic outflow tracts, and superior and inferior vena cava.1,11 M-mode measurements of the diastolic biventricular inner dimension, diastolic left ventricular internal dimension, and mitral valve excursion were obtained as previously described (Fig. 1).4...

The real-time-directed M-mode recording of the aortic root and valve was obtained by orienting the M-mode cursor (ATL Mark III, Bellevue, Washington) perpendicular to the outflow tract at the level of the semilunar valves. The two real-time views from which the M-mode was recorded were the short axis of the outflow tract and the left parasternal view (Fig. 2).

All real-time and M-mode examinations were stored on videotape. During each examination selected M-mode segments were recorded at 75 mm/sec on 6-inch hard-copy paper. An acceptable hard-copy recording for M-mode analysis consisted of the aortic valve leaflets opening and closing in their characteristic boxlike manner (Fig. 3). Ar image of the aortic root, without semilunar valve motion, was not used for analysis.

With the computer program previously described, measurements consisted of the following: aortic root dimension at end-diastole, aortic valve opening excursion, left ventricular ejection time, and heart rate (Fig. 3).

Statistical evaluation of the data (STATPRO, Boston, Massac rusetts) consisted of correlation and polynomial regress on analysis (first to fourth power). The predicted mean and the 5% and 95% confidence limits were derived for the aortic root dimension and aortic valve opening excursion for each of the fetal growth parameters (biparietal diameter, head circumference, abdominal circumference, femur length) and ventricular parameters (diastolic biventricular inner dimension, diastolic left ventricular internal dimension, mitral valve excursion).



SHORT AXIS

LEFT PARASTERNAL

Fig. 2. Real-time images illustrating the position of the M-mcde cursor for recording the aortic root dimension and valve excursion. The schematic illustrates the planes through the heart from which the real-time views were obtained. SVC = Superior vena cava; RA = right atrium; A,Ao = aorta; PA = pulmonary artery; LA = left atrium; LV = left ventricle; RV = right ventricle; <math>m = mitral valve.

Results

Regression analysis demonstrated that the best fit of the data was a first-order linear equation:

[Y(ARD, AVE =

b + mX(BPD, HC, AC, FL, DBID, LVID, MVE)]

where ARD = aortic root dimension, AVE = aortic valve excursion, BPD = biparietal diameter, HC = head circumference, AC = abdominal circumference, FL = femur length, DBID = diastolic biventricular inner dimension, LVID = diastolic left ventricular internal dimension, MVE = mitral valve excursion, m is a constant, b is the coefficient (Table I).

Aortic root dimension versus noncardiac growth measurements. There was a significant correlation (p < 0.001) between aortic root dimension and the biparietal diameter (r = 0.92), head circumference (r = 0.92), abdominal circumference (r = 0.95), and femur length (r = 0.94) (Table I). Fig. 4 illustrates the mean and the ninety-fifth and fifth confidence limits for aortic root dimension as predicted from the biparietal diameter, head circumference, abdominal circumference, and femur length.

Aortic root dimension versus ventricular measurements. There was a significant correlation (p < 0.001) between the aortic root dimension and the diastolic biventricular inner dimension (r = 0.94), diastolic left ventricular internal dimension (r = 0.92), and mitral valve excursion (r = 0.87) (Table I). Fig. 5 illustrates

the mean and the fifth and ninety-fifth confidence limits for aortic root dimension predicted from the diastolic biventricular inner dimension, diastolic left ventricular internal dimension, and mitral valve excursion.

Aortic valve excursion versus noncardiac growth measurements. There was a significant correlation (p < 0.001) between aortic valve excursion and the biparietal diameter (r = 0.85), head circumference (r = 0.85), abdominal circumference (r = 0.87), and femur length (r = 0.87) (Table I). Fig. 6 illustrates the mean and the fifth and ninety-fifth confidence limits between aortic valve excursion as predicted from the biparietal diameter, head circumference, abdominal circumference, and femur length.

Aoric valve excursion versus ventricular growth measurements. There was a significant correlation between aortic valve excursion and the diastolic biventricular inner dimension (r = 0.82), diastolic left biventricular internal dimension (r = 0.78), and mitral valve excursion (r = 0.80) (Table I). Fig. 7 illustrates the mean and the fifth and ninety-fifth confidence limits for aortic valve excursion as predicted from each of the above parameters.

Left ventricular ejection time versus heart rate. There was not a significant correlation between heart rate and left ventricular ejection time over the range studied (heart rate 121 to 181 bpm) (r = -0.1736; p > 0.10). The mean heart rate was 146 ± 25 bpm (2SD) and the mean left ventricular ejection time was 0.170 ± 0.06 (2SD).

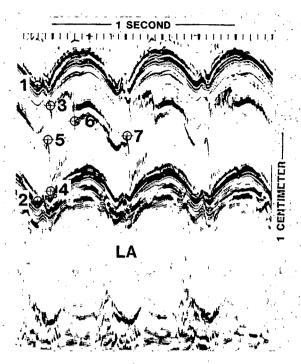


Fig. 3. M-mode tracing of aortic root and valve. The aortic M-mode measurements were measured between the following points: aortic root dimension (I and I), aortic valve excursion (I and I), left ventricular ejection time (I and I), heart rate (I and I). I0 LA = Left atrium.

Comment

A brief review of fetal circulation is important to a better understanding of the practical application of the data from this article. Initially blood enters the right atrium from the superior and inferior vena cava where a major portion from the latter flows across the foramen ovale into the lower-pressured left atrium. During ventricular diastole blood from the left atrium enters the left ventricle where it is ejected during ventricular systole through the aortic valve, into the ascending aorta. The major volume of blood from the ascending acrta is distributed to the fetal heart, head, and upper extremities.

Blood from the right atrium flows through the tricuspid valve into the right ventricle. During ventricular systole it is ejected through the pulmonary valve into the pulmonary outflow tract where a major portion flows through the ductus arteriosus into the descending aorta. From here it is distributed to the fetal trunk and placenta. Therefore, as a result of fetal circulation, the right and left ventricles function as a parallel system, that is, the right ventricle pumps blood to the body and placenta while the left ventricle pumps blood to the fetal heart, head, and upper extremities.¹²

As a result of the in utero parallel circulation, ob-

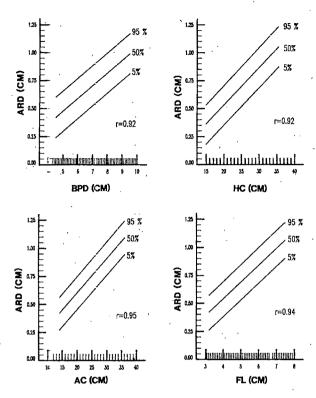


Fig. 4. Confidence limits of diastolic root dimension for noncardiac growth measurements. ARD = Aortic root dimension; BPD = Diparietal diameter; HC = head circumference; AC = abdomi.aal circumference; FL = femur length.

struction of blood flow at the level of the inflow or outflow tracts of one ventricle is often reflected by changes in the size of ventricular and outflow tract dimensions at birth. Therefore, when an anomaly of the car-liovascular system is suspected in utero, not only is it imperative to examine the structural relationships of the inflow and outflow tracts with real-time ultrasound, but also one must quantitate the dimensions of these structures. ^{1,4}

The data from this study demonstrate a high correlation (r = 0.92-0.95) between a ortic root dimension and neneardiac growth measurements (biparietal diameter head circumference, abdominal circumference, femur length). Recent studies have been published in which ultrasound-derived fetal weight or age has been correlated with real-time (r = 0.66) and Mmode measurements (r = 0.90-0.91) of the aortic root dimension (Table II).5, 17, 18 An interpretation of the meaning of the differences between the correlation coefficients from these studies is best reflected by the r'2 (correlation coefficient '2) which is the proportion of the variance of Y (aortic root dimension) that can be predicted from the variance of X (weight, age, growth measurements). Thus, if r = 0.95 for abdominal circumference and aortic root dimension, we can say that

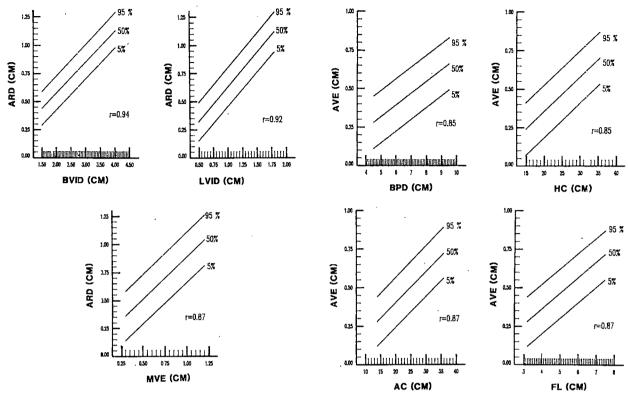


Fig. 5. Confidence limits of aortic root dimension for cardiac ventricular measurements. ARD = Aortic root dimension; BVID = biventricular inner dimension—diastole; LVID = left ventricular internal dimension—diastole; MVE = mitral valve opening excursion.

Fig. 6. Confidence limits for aortic valve excursion for noncardiac growth measurements. AVE = Aortic valve excursion;BPD = biparietal diameter; HC = head circumference; AC =abdominal circumference; FL = femur length.

Table I. Correlation and linear regression analysis comparing M-mode measurements of the aortic valve with noncardiac and cardiac growth parameters (N = 43)

		Correlation and	ılysis		Regression	ı analysis	
	r	r'2	p Value	Standard error estimate	F test	m	ь
Aortic root	dimension (Y)),					- · · · -
with $X =$							
BPD	0.92	0.85	< 0.001	0.087	216*	0.1145	-0.0916
HC	0.92	0.85	< 0.001	0.085	234*	0.0339	-0.1476
AC	0.95	0.90	< 0.001	0.071	356*	0.0306	-0.0058
FL	0.94	0.88	< 0.001	0.077	302*	0.1498	-0.0549
BVID	0.94	0.88	< 0.001	0.071	270*	0.2788	0.0233
LVID	0.92	0.85	< 0.001	0.081	205*	0.6134	0.0161
MVE	0.87	0.75	< 0.001	0.104	108*	0.7520	0.1331
Aortic valve	excursion (Y),					
with $X =$	•	,					
BPD	0.85	0.71	< 0.001	0.083	104*	0.0756	-0.0540
HC	0.85	0.71	< 0.001	0.082	111*	0.0224	-0.0920
\mathbf{AC}	0.87	0.76	< 0.001	0.077	130*	0.0202	0.0036
FL	0.87	0.76	< 0.001	0.077 .	130*	0.0995	-0.0325
BVID	0.82	0.67	< 0.001	0.086	74*	0.1751	0.0388
LVID	0.78	0.60	< 100.0>	0.094	54*	0.3707	0.0497
MVE	0.80	0.63	< 0.001	0.090	62*	0.4967	0.0936

Y = mX + b; Y = aortic dimension; X = growth or cardiac measurement. BPD = Biparietal diameter; HC = head circumference; AC = abdominal circumference; FL = femur length; DBID = diastolic biventricular inner dimension; LVID = left ventricular internal dimension; MVE = mitral valve excursion. p < 0.0001.

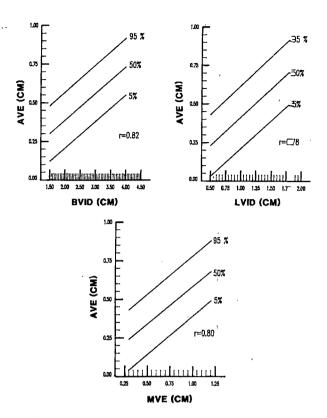


Fig. 7. Confidence limits for aortic valve excursion for cardiac ventricular measurements. AVE = Aortic valve excursion; BVID = biventricular inner dimension—diastole; LVID = bit ventricular internal dimension; MVE = bitral valve opening excursion.

90% (r'2 × 100) of the variance of one variable (a) rtic root dimension) is predictable from the variance of the other variable (abdominal circumference). In effect we know 90% of what we would have to know to make a perfect prediction of one variable from the other. With the use of r'2, it can be seen that the M-mode neasurements of a ortic root dimension (r'2 = 0.85 to 1.90) are much higher than the real-time measurements previously reported (r'2 = 0.43) (Table II). 17

Besides the difference in r'2 values, there was ε significant difference (p < 0.05) between the correl_tion coefficients (Z > 1.96) of the current study and thepreviously reported real-time and M-mode cross-sectional studies (Table II).^{5, 17, 18} These findings are most Lkely secondary to differences in imaging techniques since the correlation coefficients between aortic root dimension and fetal weight (r = 0.93) and gestational age (r = 0.92) from our data base are not different from those of the growth measurements (biparietal diameter, head circumference, abdominal circumference, from length) (G. R. DeVore, B. Siassi, and L. D. Platt unpublished data).

Although previous studies have reported the cortic root dimension, this is the first study to include cortic valve excursion. The importance of aortic valve excur-

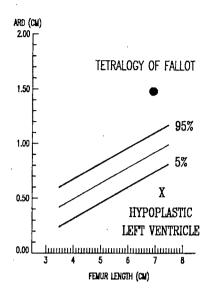


Fig. 8. A ortic roct dimension in fetuses with a hypoplastic left ventricle (X) and tetralogy of Fallot (\bullet) . ARD = A ortic root dimension.

sion is that it allows the clinician to assess the excursion of the valve as well as to provide an internal marker from which to determine if the plane of the M-mode is correctly oriented through the center of the aortic root. If either the anterior or posterior leaflets are not imaged, then the possibility exists of cutting through the root tangentially, thus foreshortening or increasing the actual aortic root dimension. Imaging the aortic valve excursion also enables left ventricular ejection time to be measured. As mentioned, the left ventricular ejection time was not correlated with heart rate during the intervals studied. This suggests that under normal physiclogic conditions the length of the ejection phase does not vary with heart rate.

The second part of the data analysis was the determinat on of confidence limits (5% and 95%) for the predicted Y (aortic root dimension, aortic valve excursion) from a given X (biparietal diameter, head circumference, abdominal circumference, femur length, diastolic piventricular inner dimension, diastolic left ventricular internal dimension, mitral valve excursion). The benefit of this type of analysis is that measurements from Tetuses at risk for congenital anomalies (tetralogy of Falot, aortic stenosis) can be compared with normal data to ascertain whether the aortic root dimension and aortic valve excursion are abnormal. To accomplish this, regression analysis was used for purposes of predicting aortic root dimension from a given fetal noncardiac measurement (biparietal diameter, head circumference, abdominal circumference, femur length) and a cardiac (diastolic biventricular inner dimension, diastclic left ventricular internal dimension, mitral valve excursion) growth measurement. This type of analysis, however, requires that each data pair (bipa-

Table II. Comparison of aortic root diameter with published studies

Study	Real-time measurement	M-mode measurement	r	r'2	Z	Standard error of estimate
This study						
BPD '		X	0.92	0.85		0.088
AC		X	0.95	0.90		0.070
Allan et al.,5 gestational age*		X	0.90	0.81	p < 0.05	0.069
Sahn et al., 17 estimated fetal weight†	X		0.66	0.44	p < 0.05	Not given

Z = Comparison of this study with those of Allan et al. and Sahn et al.

rietal diameter, adrtic root dimension) be entered only once for each fetus studied. Thus, longitudinal studies in which the same fetus is examined more than once cannot use regression analysis.18 For this reason a crosssectional study was undertaken which demonstrated that a first-order linear equation best described the data.

By regressing the echocardiographic data against measured fetal growth parameters (biparietal diameter, head circumference, abdominal circumference, femur length) the examiner can choose which noncardiac growth measurement to use. This is especially important when there is an abnormal cephalic index or when one is dealing with a fetus with an anomaly of the fetal head, abdomen, or extremities in which one of the above growth measurements cannot be used because it is abnormal. 19-21 For example, if the fetal cardiovascular system of a fetus with hydrocephalus or dwarfism is evaluated, one should use the corresponding confidence limit graph which does not involve the affected organ system.

Comparison of aortic root dimension and aortic valve excursion with cardiac structures (diastolic biventricular inner dimension, diastolic left ventricular internal dimension, mitral valve excursion) allows quantitative examination of cardiac interrelationships. Pathologic conditions of the left ventricle and/or mitral valve might reflect changes in the size of the aortic valve and/or aortic root, that is, aortic stenosis, tetralogy of Fallot, hypoplastic aortic arch. Therefore, understanding the relationships between the aortic root dimension (aortic valve excursion) and ventricular structures could be quite useful when one is assessing inflow and outflow tract measurements in fetuses with suspected cardiac

Although the subject of future reports, the approach outlined in this paper has assisted us in the diagnosis of fetuses with aortic stenosis, hypoplastic left ventricle, tetralogy of Fallot, and truncus arteriosus (Fig. 8).

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^{*}Gestational age determined from the biparietal diameter.

[†]Estimated fetal weight determined from the biparietal diameter and abdominal circumference.

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Maternal serum α -fetoprotein in twin pregnancies uncomplicated by neural tube defect

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The maternal serum α -fetoprotein concentration was measured between 16 and 20 weeks in 145 twin pregnancies in which neither fetus had a neural tube defect. When the maternal serum α -fetoprotein concentration was less than two multiples of the singleton median, pregnancy outcome was good; the extended perinatal mortality rate was 32.6/1000, mean birth weights for the first and second twins were 2507 and 2443 gm, respectively, and mean gestation at delivery was 36 weeks, 6 days. When the maternal serum α -fetoprotein concentration was greater than four multiples of the singleton median, the outcome was poor; the extended perinatal mortality was 400/1000, mean Eirth weights were 1963 and 1523 gm, and mean gestation at delivery was 32 weeks, 4 days. The negative correlations of maternal serum α -fetoprotein concentration with birth weight and gestation at delivery were highly significant. Maternal serum α -fetoprotein concentration in midpregnancy is a useful predictor of outcome in twin pregnancy, independent of the occurrence of neural tube defect, and it appears to be related to the timing of delivery rather than fetal growth. (AM J OBSTET GYNECOL 1985;152:550·3.)

Key words: α-Fetoprotein, twins, perinatal mortality, neural tube defect, high-risk pregnancy

Elevated maternal serum α -fetoprotein concentration is a sensitive indicator of fetal neural tube defect, 1.2 but it also occurs in several other circumstances, including threatened or missed abortion, multiple pregnancy, and other fetal abnormalities, particularly abdominal wall defect. 3 Even in the absence of any of these conditions, an elevated maternal serum α -fetoprotein concentration is a high-risk factor. Intrauterine growth retardation or preterm delivery will result in a low-birth weight baby in up to 35% of cases, and perinatal mortality is increased threefold. 4.6

In twin pregnancy the median maternal serum α -fetoprotein level is similar to the upper limit of normal for singleton infants at two to two and a half multiples of the singleton median.^{7,8} Ghosh et al.⁸ suggested that five multiples of the singleton median is a suitable cutoff point in twin pregnancy because it was exceeded by all 11 twin pregnancies complicated by neural tube defect in their series. They also showed that twin pregnancies

with maternal serum α-fetoprotein greater than five multiples of the singleton median had a fetal loss rate exceeding 50%, intrauterine death being the mechanism most commonly involved.

Ghosh et al.⁸ and Wald et al.⁷ noted a negative correlation between the maternal serum α -fetoprotein level and birth weight in twins, but they were unable to ascertain whether earlier delivery or impaired fetal growth was mainly responsible.

In the present study we examined the prognostic significance of maternal serum α -fetoprotein levels in a consecutive series of twin pregnancies with delivery in a large British city during a 2-year period.

Material and methods

Twin births in Glasgow, Scotland, during 1981 and 1982 were ascertained from statutory birth notification records. The maternal and infant case records of all 283 twin pairs delivered in the city's five major obstetric units were studied, and data were collected on maternal serum α-fetoprotein level, ascertainment of gestational age, pregnancy complications, gestation at delivery, birth weight, and weight for gestational age. Ultrasound is routinely used to confirm gestational age at the first attendance in three of the five hospitals and

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Table I. Maternal serum α -fetoprotein and birth weight, gestational age at delivery, intrauterine growth retardation, and pregnancy outcome

	Mai	ernal serum α-fetoprote	in level (MOM) at 16-20	0 wk
	<2	2-<3	3-<4	`≥4
No. of pregnancies	46	68	21	10
Birth weight				
Mean (twin 1) (gm ± SD)	$2507* \pm 618$	2279 ± 659	$2192* \pm 541$	1963 ± 975
Mean (twin 2) (gm ± SD)	$2443* \pm 566$	2319 ± 659	2153 ± 579	$1523* \pm 984$
<2500 gm (%)	46.7	58.8	76.2	75.0
<1500 gm (%)	6.5	10.3	7.1	55.0
Gestational age at birth				
Mean (days \pm S.D.)	$258*† \pm 17.5$	251 ± 22.6	$245* \pm 22.7$	$228\dagger \pm 35.7$
<37 wk (%)	39.1	52.9	71.4	60.0
≤34 wk (%)	10.8	23.5	28.6	50.0
Intrauterine growth retardation				
<fifth (%)<="" percentile="" td=""><td>22.7</td><td>16.4</td><td>25.0</td><td>25.0</td></fifth>	22.7	16.4	25.0	25.0
<tenth (%)<="" percentile="" td=""><td>39.8</td><td>33.6</td><td>36.1</td><td>25.0</td></tenth>	39.8	33.6	36.1	25.0
Pregnancy outcome				
Stillbirth	2	8	1	4 .
First-week death	0	- 4	0	2
Postperinatal death	1	0	0	4 . 2 2 8
Total deaths	3	12	1	8
Extended perinatal mortality rate (No./1000)	32.6	88.2	23.8	400.0

MOM = Multiples of singleton median.

very liberally used in the other two; 83% of all the twin pregnancies had been identified and gestational age confirmed by 24 weeks, and only 5.3% were first recognized after the onset of labor. The Aberdeen birth weight standards of Thomson et al.9 were used, and babies weighing less than the fifth percentile for gestational age were classified as intrauterine growth retarded.

Maternal serum α-fetoprotein was measured between 16 and 20 weeks' gestation in 145 twin pregnancies, none of which was complicated by neural tube defect. Amniocentesis was not performed in any of the cases. Maternal serum a-fetoprotein levels were reported as multiples of the singleton median, so that values at different gestational ages between 16 and 20 weeks could be compared.

Perinatal deaths included babies stillborn after 28 weeks' gestation and live-born babies of any gestational age and birth weight dying in the first 7 days of life. Deaths occurring after the first week where the cause was perinatally related,10 being due to either prematurity or congenital abnormality, were also studied. Such postperinatal deaths combined with the perinatal deaths were described as the "extended perinatal mortality rate."

Results

The maternal serum α -fetoprotein level was less than two multiples of the singleton median in 46 cases (31.7%), between two and three in 68 (47.2%), between

Table II. Maternal serum α -fetoprotein level and the frequency of spontaneous premature rupture of the membranes in cases of preterm labor

	Maternal serum α-fetoprotein (MOM)						
	<2	2-<3	3-<4	≥4			
Preterm labor (<37 wk)	18	36	15	7			
Premature rupture of membranes in cases of preterm labor	2 (11%)	9 (25%)	8 (53%)	3 (43%)			

MOM = Multiples of singleton median.

three and four in 21 (14.6%), and greater than four in 10 (6.9%). There was a highly significant correlation between maternal serum α-fetoprotein and birth weight, which was somewhat greater for the second twin (r = -0.367, p < 0.001) than for the first (r =-0.254, p < 0.01). The mean birth weights and the incidences of birth weight <2500 and 1500 gm at different maternal serum α-fetoprotein levels are shown in Table I.

There was also a highly significant negative correlation between maternal serum α -fetoprotein levels and gestation at delivery (r = -0.354, p < 0.001). The mean gestation at delivery and the incidences of delivery before 37 and 34 weeks at different maternal serum α-fetoprotein levels are also shown in Table I.

^{*}p < 0.05.

tp < 0.01.

Table III. Twin pregnancies in which maternal se um α -fetoprotein exceeded 4 multiples of singleton median

	in 2	Tw	Twis 1			Maternal serum	
Comments	Outcome	W∹ight (⊊m)	Outcome	Weight (gm)	Gestation at birth (days)	α-fetoprotein level (MOM)	No.
Y WARRANT TO THE PARTY OF THE P	s ·	1400	S	1780	212	4.2	1
	SB	1:000	S	2810	262	4.2	2
Antepartum hemorrhage	S	2580	S	2380	250	4.3	3
Antepartum hemorrhage	. S .	1030	S	1130	198	5.1	4
1 0	S	3540	S	3320	269	5.3	5
Acute hydramnios; feto- fetal transfusion	SB ,	1100	SB	0840	200	5.3	6
	S .	2240	S	2320	232	5.6	7
Twin 2, intrauterine death at around 31 wk	SB	0525	S	3200	283	6.0	8
SROM	NND	0880	NND	0840	192	6.1	9
SROM	NND	0840	NND	1010	186	6.6	10

MOM = Multiples of singleton median; S = survival; SB = stillbirth; NND = neonatal death; SROM = spontaneous premature rupture of the membranes.

In contrast, different levels of maternal serum afetoprotein were not associated with any significant change in the incidence of intrauterine growth retardation, expressed as the percentage of babies weigning less than the singleton fifth or tenth percentile for gestational age.

There was no difference in the incidence of pregnancy hypertension or antepartum hemorrhage a different maternal serum α -fetoprotein levels, and other pregnancy complications occurred too infrequently for meaningful analysis. It is, however, interesting to note that, when preterm labor occurred, spontaneous rupture of the membranes was a more common mode of onset at higher maternal serum α -fetoprotein evels (Table II).

The outcome of pregnancy was worse with higher maternal serum α-fetoprotein levels, the extended perinatal mortality rate rising from 32.6/1000 where maternal serum α-fetoprotein had been below two multiples of the singleton median to 400/1000 where it had exceeded four. The extended perinatal mortality among all twins in Glasgow during the study regriod was 83/1000. Individual details of the 10 pregnencies in which maternal serum α-fetoprotein exceedec four multiples of the singleton median are shown in Table III. Although eight of the 20 babies (40%) died, no common pathologic conditions underlay the poct outcome in this group, with unexplained intrau_erine death, antepartum hemorrhage, spontaneous premature rupture of the membranes, and fetofetal gransfusion all occurring. In seven pregnancies with maternal serum α-fetoprotein exceeding five multiples of the singleton median, half the babies died, as did ave of six babies in the three pregnancies in which mæernal serum α-fetoprotein exceeded six multiples of the singleton median.

Comment

The results confirm that the presence of elevated maternal serum α -fetoprotein levels at 16 to 20 weeks in a twin pregnancy not complicated by neural tube defect or other fetal anomaly is a high-risk factor and that the higher the maternal serum α -fetoprotein level, the worse the pregnancy outcome.

Although only 50% of twins had maternal serum α -fetoprotein measured during the study period, we do not believe that selection bias in any way affected the results for two principal reasons. First, the overall extended perinatal mortality rate was identical in the screened and unscreened groups (82.7/1000 versus 83.3/1000), and second, no information was then available to suggest a particular role for maternal serum α -fetoprotein in complicated twin pregnancies. Indeed, a commonly held view at that time was that maternal serum α -fetoprotein measurement should be omitted in multiple pregnancies because of less easy interpretation in the diagnosis of neural tube defects, and many of the results reported here were obtained "accidently" in pregnancies in which the twins were hitherto undiagnosed.

This worsening outcome with higher maternal serum α-fetoprotein levels seems to be mediated principally by earlier delivery rather than impaired fetal growth. This is a somewhat unexpected finding since if an elevated maternal serum α-fetoprotein level, in the absence of fetal abnormality, is a marker of fetomaternal hemorrhage¹² or a "leaky placenta," it would seem more likely to affect fetal growth or be associated with placental separation rather than to influence the onset of labor. As in singleton pregnancy, ^{5, 6} elevated maternal serum α-fetoprotein in twins is not associated with any spec fic pregnancy complication, but in contrast to singleton pregnancies, it is a more specific and selective

predictor of poor pregnancy outcome. Thus, in singleton pregnancies with maternal serum α-fetoprotein levels greater than two and a half multiples of the singleton median, only about 35% develop a pregnancy complication and the perinatal mortality is 3% to 4%,6 whereas among the twin pregnancies studied, a maternal serum α-fetoprotein level greater than four multiples of the singleton median was followed by an uncomplicated pregnancy in only 10% and the perinatal mortality was as high as 40%; 33% of all the perinatal deaths occurred in the 7% of twin pregnancies with maternal serum αfetoprotein levels greater than four multiples of the singleton median.

These results suggest that maternal serum α-fetoprotein is a valuable screening test in twin pregnancy and could usefully be introduced even in regions where there is a low incidence of neural tube defect and even when the parents do not request neural tube defect screening. Twin pregnancies with maternal serum αfetoprotein greater than four multiples of the singleton median and uncomplicated by neural tube defect should be monitored carefully, and referral to a perinatal center may enable measures such as ultrasonic fetal weight estimation,12 nonstress cardiotocographic testing,18 and optimum management of preterm labor to improve the poor outcome in this group of pregnancies.

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Sympathoadrenal and cardiovascular reactivity in pregnancyinduced hypertension

II. Responses to tilting

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Sympathoadrenal and cardiovascular responses to tilting were studied in patients with pregnancy-induced hypertension and healthy control subjects during the last trimester of pregnancy and 8 to 12 weeks post partum. Blood volumes were lower in the patients with pregnancy-induced hypertension during pregnancy (0.065 versus 0.081 L/kg, p < 0.01) but not post partum. Tilting induced significantly smaller increases in heart rate and arterial plasma norepinephrine concentrations and smaller changes in blood pressure during pregnancy as compared to after pregnancy in both groups. Forearm vascular resistance increased significantly in both groups after pregnancy but only in the patients with pregnancy-induced hypertension during pregnancy. The forearm vasoconstrictor response to tilting was, in fact, totally abolished in the third trimester of normal pregnancy. The hypertensive patients had higher arteria plasma epinephrine levels at rest and greater epinephrine and norepinephrine responses to tilting than the control subjects during pregnancy. Normal pregnancy appears to reduce the circulatory and sympa hoadrenal responses to orthostatic stress, presumably because of volume expansion that allows ver ous return to be better maintained in the upright position. The less pronounced pregnancy-induced increase in blood volume in patients with pregnancy-induced hypertension appears to explain the increased sympathoadrenal and forearm vascular reactivity in this group during pregnancy. (AM J OBSTET GYNECOL 1985;152:554-60.)

Key words: Arterial plasma norepinephrine, epinephrine, blood volume, orthostatic stress, forearm blood flow

During orthostatic stress, for example, that induced by tilting, there is an abrupt reduction of venous return to the heart that triggers an increase in heart rate and peripheral vasoconstriction, both of which serve to maintain blood pressure in the upright position. ¹⁻³ This normal hemodynamic response to tilting is accompanied by rapid increases in arterial plasma norepinephrine and epinephrine concentrations. ⁴ In fact, the plasma catecholamine response to orthostatic provocation is a commonly used test of sympathetic function in normotensive and hypertensive subjects. ⁵

The present study was performed to evaluate sympathoadrenal and cardiovascular reactivity to orthostatic provocation in patients with pregnancy-induced

hypertension. Responses to orthostatic stress are, regardless of the provocation technique (standing, passive tilting, or lower body negative pressure), to a considerable extent dependent on the inhibition of volume receptors on the low-pressure side of the circulation. In normal pregnancy there is a considerable increase in blood volume, which may counteract the decrease in venous return and lead to less pronounced counterregulatory responses during orthostatic stress. In pregnancy-induced hypertension, however, the pregnancy-induced increase in blood volume is less pronounced, and therefore the counterregulatory responses of these patients during orthostatic stress may be greater than in normal pregnancy.

In the present study we have examined blood volumes and cardiovascular and sympathoadrenal responses of patients with mild pregnancy-induced hypertension and matched healthy pregnant control subjects curing an orthostatic provocation induced by head-up tilting. To evaluate the effect of pregnancy per se, the subjects were also studied 8 to 12 weeks post partum. Sympathoadrenal activity was evaluated by determinations of arterial plasma catecholamine concentrations. Cardiovascular variables included intra-arterial blood pressure, heart rate, and forearm

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Table I. Clinical data for the pregnancy-induced hypertension group

			Body we	Body weight (kg)		`	Blood		Apgar score		
Patient No.	Age (yr)	Height (cm)	Before pregnancy	During pregnancy	Parity	Gestational length (wk)	pressure at admission (mm Hg)	Protein- uria	1 min	5 min	Fetal weight (gm)
1	29	163	55	70	1	. 35	160/90	+	9	10	2500
2*	31	169	74	89	2	37	150/90	_	9	10	2860
3	27	169	58	75	0	39	140/100	+	9	10	2980
4	25	165	55	74	0	40	150/95	_	8	10	4 020
5	31	162	71	87	1	32	145/100	_	9	10	3820
6	27	166	60	70	0	40	145/105	_	8	10	2720
7	30	156	58	71	0	35	145/90	+	1	3†	740
8	32	173	95	123	0	38	150/90	+	9	10	4310
9	29	173	82	92	0	40	145/100	+	7	9	2560
10*	26	168	58	76	0	38	145/95	+	9	10	3470
11	32	166	73	87	1	39	160/105	+	9	10	3770
Mean	29	166	66	82		37.4	149/96				2835
95% Confidence interval	27-32	163-170	58-75	73-92		35.7-39.3	144-153/ 92-100				2048-3924

^{*}Patient did not participate in the postpartum investigation.

Table II. Clinical data for the control group

Patient No.	Age (yr)		Body weight (kg)				Apgar score		
		Height (cm)	Before pregnancy	During pregnancy	Parity	Gestational length (wk)	l min	5 min	Fetal weight (gm)
1	25	168	63	77	0	40	9	10	3520
2	28	166	75	93	0	38	9	10	3890
3*	19	161	66	74	0	37	8	10	4040
4	27	168	63	82	0	37	10	10	3670
5	29	170	59	73	0	38	9	9	4050
6*	21	175	56	69	0	36	9	10	3630
7	28	180	67	79	0	36	10	10	3080
8	31	165	57	69	3	39	9	10	3440
Mean	26	169	63	77		37.6			3652
95% Confidence interval	22-30	164-174	58-68	· 71-83		36.4-38.8			3382-3943

^{*}Patient did not participate in the postpartum investigation.

blood flow. This investigation was performed in connection with a study of responses to somatomotor activation induced by an isometric handgrip test and a cold pressor test in the same subjects. [Nisell H, Hjemdahl P, Linde B, Lunell N-O. Sympathoadrenal and cardiovascular reactivity in pregnancy-induced hypertension. Responses to isometric exercise and a cold pressor test (in preparation)].

Material and methods

Subjects. The hypertensive group consisted of 11 previously normotensive patients with mild pregnancy-induced hypertension, defined as blood pressure exceeding 140/90 mm Hg on two occasions more than 6 hours apart during the last trimester (Table I). The control group consisted of eight healthy pregnant

women with pregnancies of the same duration (Table II). The studies were performed twice—during the last trimester of pregnancy and 8 to 12 weeks post partum. On the second occasion nine patients with pregnancy-induced hypertension and six control subjects participated in the tilt test. For further details concerning other studies in these subjects see the forthcoming paper mentioned above. All subjects gave their informed consent to participate in the study, which was approved by the Ethical Committee at Huddinge University Hospital.

Methods. Arterial blood pressure was measured invasively in a brachial artery and heart rate was monitored by electrocardiogram. Forearm blood flow was measured by venous occlusion plethysmography. Forearm vascular resistance was calculated by dividing the

[†]Died at birth.

Table III. Values preceding the tilt procedure (geometric mean values; 95% confidence interval within parentheses)

	Pregnancy-induced hypertension, pregnant	Pregnancy-induced hypertension, post partum	Control group, pregnant	Control group, post partum	p Value
Systolic blood pressure (mm Hg)	136.4 (133.2-139.6)	127.1 (123.9-130.3)	113.0 (109.3-116.7)	115.0 (111.3-118.7)	*, †
Diastolic blood pressure (mm Hg)	88.6 (86.0-91.2)	81.5 (78.9-84.1)	72.7 (69.7-75.7)	72.8 (69.8-75.8)	†
Heart rate (bpm)	75.3 (72.1-78.7)	68.6 (65.6-71.7)	73.7 (69.8-77.8)	63.2 (59.9-66.8)	±
Forearm vascular resistance (PRU)	193 (160-233)	186 (154-225)	269 (219-331)	241 (196-296)	NS
Norepinephrine (nmol/L)	1.07 (0.99-1.17)	0.75 (0.69-0.81)	1.19 (1.07-1.31)	1.04 (0.94-1.16)	§ .
Epinephrine (nmol/L)	0.36 (0.31-0.41)	0.21 (0.17-0.25)	0.08 (0.04-0.12)	0.21 (0.16-0.26)	§,

NS, Not significant.

mean arterial pressure by the forearm blood flow and was expressed in arbitrary units (peripheral resistance units). The plasma volume was determined by dye dilution, with Evans blue used as indicator. The blood volume was calculated as plasma volume \div $(1-0.91\times\text{hematocrit})$, where the hematocrit was measured in a peripheral vein and 0.91 is the correction factor used for calculations of whole-body hematocrit. Arterial plasma catecholamine concentrations were measured by cation-exchange high-performance liquid chromatography with electrochemical detection. For more detailed information see the forthcoming paper mentioned.

Procedure. This investigation was performed in connection with the experiments described in the paper above-mentioned in all but one woman. The women were lying in the left lateral recumbent position at a 30-degree angle in order to reduce the effects of the pregnant uterus on venous return. A resting period of 25 minutes was allowed after the cold pressor test before the first of two basal measurements (10 minutes apart) was made. The means of these two measurements were used in calculations. During the resting period blood volume was determined by the equation described above. Immediately after the second basal measurement an orthostatic provocation was performed by passively tilting the woman to 70 degrees (head up) during 6 minutes. Arterial blood pressure was recorded continuously with interruptions for blood sampling at 2, 4, and 6 minutes for determinations of arterial plasma catecholamine concentrations. Heart

rate was monitored continuously and forearm blood flow measured at approximately 30-second intervals.

Statistical analysis. The statistical method used to compare circulatory and hormonal variables between the two groups as well as between the two occasions (a four-way analysis of variance described in detail in the forthcoming paper) allowed inclusion only of the 15 subjects participating twice. The remaining four subjects (indicated by parentheses in Tables I and II) were included only in calculations of possible correlations between circulatory and hormonal variables. Their responses were, however, similar to those found in the remaining subjects in the respective group.

Briefly, a four-way analysis of variance was used after transformation of data to logarithmic values in order to obtain a normally distributed material. Blood pressure values were not transformed, since they were approximately normally distributed. In selected cases (see the forthcoming paper) the Wilcoxon matched pairs signed-ranks test or the Mann-Whitney U test were used. To study possible correlations between circulatory and hormonal variables, the Spearman rank correlation test was used.

All results in the text, tables, and figures are given as geometric means. In the tables, the 95% confidence intervals for the means are also given.

Resu ts

Resting conditions. It should be noted that approximately 2 hours elapsed from the time of catheterization until the "basal" measurements preceding the tilt prov-

^{*}Pregnancy-induced hypertension, pregnant, versus pregnancy-induced hypertension, post partum: p < 0.05.

[†]Pregnancy-induced hypertension versus control group: p < 0.01 at both examinations.

[‡]Control group, pregnant, versus control group, post partum: p < 0.05.

Pregnancy-induced hypertension, pregnant, versus pregnancy-induced hypertension, post partum: p < 0.05.

^{||}Pregnancy-induced hypertention, pregnant, versus control group, pregnant: p < 0.01.

4.26 (4.07-4.46)

0.064 (0.057-0.071)

*, †

Blood volume (L)

volume (L/kg)

Relative blood

Pregnancy-induced Pregnancy-induced hypertension, hypertension, Control group, Control group, pregnant nonpregnant nonpregnant pregnant b Value

Table IV. Absolute and relative blood volumes (geometric mean values; 95% confidence interval within parentheses)

3.99 (3.45-4.60)

0.056 (0.049-0.064)

ocation. This and the fact that not all subjects participated in the tilt test explain some minor discrepancies between the "basal" values of the present report and those of the forthcoming paper.

5.53 (4.62-6.60)

0.065 (0.060-0.071)

Circulatory variables (Table III). Systolic and diastolic blood pressures were significantly higher in the pregnancy-induced hypertension group than in the control group both during pregnancy and post partum (p < 0.01). Systolic blood pressure decreased significantly (p < 0.05) at the postpartum examination in the pregnancy-induced hypertension group. There was a tendency toward lower heart rates at the postpartum examination in the pregnancy-induced hypertension group and a significant (p < 0.05) reduction in the control group as compared to values in the pregnant state. There was, however, no significant difference between the two groups at either occasion, in contrast to the findings in the somewhat larger material reported in the future paper. Forearm vascular resistance was not significantly influenced by either pregnancy-induced hypertension or pregnancy per se. The control subjects, however, tended to demonstrate higher values than the pregnancy-induced hypertension group on both occasions.

Arterial plasma catecholamine concentrations (Table III). During pregnancy the pregnancy-induced hypertension group exhibited significantly higher epinephrine levels than the control group (p < 0.01). In the former group the epinephrine concentration decreased significantly post partum (p < 0.05). The pregnancy-induced hypertension group demonstrated significantly higher norepinephrine levels during pregnancy than they did after pregnancy (p < 0.05), but there were no significant differences between the groups at either examination.

Blood volume (Table IV). In the pregnant state blood volume in the pregnancy-induced hypertension group was 5.53 L (geometric mean) as compared to 6.35 L in the control group. This difference was not significant. However, if body weight was taken into account, the pregnancy-induced hypertension group exhibited a significantly lower weight-related blood volume than the control group (0.065 versus 0.081 L/kg, p < 0.01).

At the postpartum examination blood volumes were s.milar in the pregnancy-induced hypertension and control groups both in absolute terms (3.99 versus 4.26 L/kg, NS) and when related to body weight (0.056 versus 0.054 L/kg, NS).

Responses to tilting

6.35 (5.35-7.53)

0.081 (0.069-0.094)

Circulatory variables (Fig. 1). Tilting caused a slight and insignificant increase in systolic blood pressure in both groups during pregnancy and a similarly slight decrease at the postpartum examination. This resulted in a significant (p < 0.05) difference in the systolic blood pressure response on the two occasions in both groups but no difference between the groups. Diastolic blood pressure increased significantly, (p < 0.01) during tilting on both occasions, the average increases ranging from 5 to 9 mm Hg. The diastolic blood pressure response was not influenced by either pregnancyinduced hypertension or pregnancy per se. The increase in heart rate during tilting ranged from 14 to 30 bpm and was significant under all conditions (p < 0.01). The heart rate responses of the two groups did not differ significantly during or after pregnancy but were greater in both groups at the postpartum examination (p < 0.01).

In the control group, tilting induced an increase in forearm vascular resistance at the postpartum examination but no vasoconstriction during pregnancy. In the pregnancy-induced hypertension group, on the other hand, there was a forearm vasoconstrictor response to tilting, which tended to be exaggerated during pregnancy. During pregnancy the pregnancy-induced hypertension group thus demonstrated a sign:ficantly greater response than the control group (p < 0.01). At the postpartum examination, however, the forearm vascular responses of the two groups did not differ significantly.

Arterial plasma catecholamine concentrations (Fig. 2). The tilt procedure caused significant (p < 0.01) increases in arterial plasma norepinephrine under all conditions. The pregnancy-induced hypertension group exhibited a significantly greater response than the control group during pregnancy (increases of 102% versus 49%, p < 0.05). The changes induced by tilting after preg-

^{*}Pregnant versus post partum: p < 0.05.

[†]Pregnancy-induced hypertension, pregnant, versus control group, pregnant: p < 0.01.

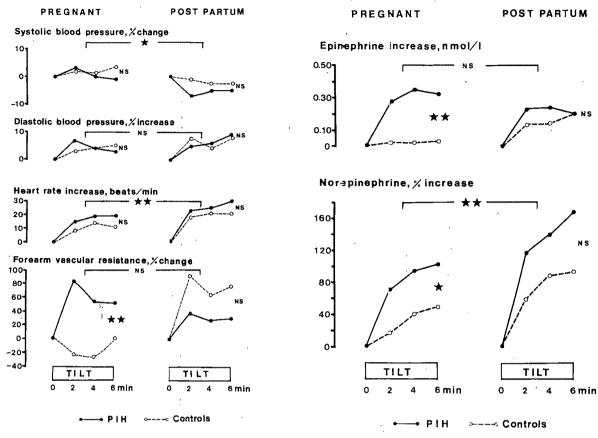


Fig. 1. Relative changes in systolic and diastolic blood pressures and forearm vascular resistance and absolute changes in heart rate during orthostatic provocation induced by passive tilting to 70 degrees (head up) during 6 minutes. All subjects were in the left lateral position (30-degree angle of rotation) to reduce the effect of the pregnant uterus on venous return in the recumbent position. Basal values are given in Table III. *p < 0.05; **p < 0.01; NS, not significant. Values for significance between the two examinations refer to possible differences between pregnant and postpartum responses in both groups.

nancy were significantly (p < 0.01) greater than those observed during pregnancy in both groups (increases of 168% in the pregnancy-induced hypertension group and 94% in the control group). In the control group the arterial plasma epinephrine concentration increased significantly (p < 0.01) only at the postpartum examination. In the pregnancy-induced hypertension group, on the other hand, the epinephrine response to tilting (a doubling of the arterial plasma levels) was significant (p < 0.01) and similar during and after pregnancy. The difference between the two groups at the pregnancy investigation was significant (p < 0.01), but the epinephrine responses after pregnancy were similar.

Correlations between circulatory variables and norepinephrine. The pregnancy-induced hypertension group exhibited a significant inverse correlation be-

Fig. 2. Relative changes in arterial plasma concentrations of norepinephrine and absolute changes of epinephrine in connectior with passive tilting. Epinephrine responses are given in absclute terms because the basal levels occasionally are so low that transformation to percent changes will give unrepresentative responses. For basal values see Table III. Symbols as in Fig. 1.

tween weight-related blood volumes and arterial plasma norepinephrine concentrations both at rest $(r=-0.59,\,p<0.05)$ and during tilting $(r=-0.70,\,p<0.05)$ during pregnancy but not in the nonpregnant state. In the control group no such correlation was seen either during or after pregnancy. There were no correlations between blood volumes and the forearm vascular resistance or norepinephrine responses to tilting or between the vascular resistance response and plasma norepinephrine responses to tilting in either group.

Comment

In healthy nonpregnant persons subjected to orthostatic stress, the mean arterial blood pressure is maintained through counterregulatory reflexes that cause increases in heart rate and peripheral vascular resistance. Passive tilting induces vasoconstriction in the fcrearm, which is absent in the sympathectomized forearm. The vasoconstrictor response of the arm to

tilting is similarly pronounced in adipose tissue and skeletal muscle and is accompanied by rapid increases in the arterial plasma concentrations of norepinephrine and epinephrine.4 Sundlöf and Wallin11 have demonstrated a stimulus intensity-dependent increase in muscle sympathetic nerve activity during negative pressure in the lower body. Using the same stimulus to cause graded reductions of venous return to the heart, Zoller et al.12 could show that forearm vascular resistance was very sensitive to this kind of stimulation, since vasoconstriction could be induced without changes in heart rate. Thus inhibition of cardiovascular low-pressure (volume) receptors is an efficient way to induce vasoconstriction in human forearm tissues. Carotid baroreceptors will, of course, also be influenced by a change in posture that alters the hydrostatic pressure in that region. Changes in arterial baroreceptor activity are, however, of less importance than changes in volume receptor activity for the vascular tone of the forearm.12

During orthostatic stress blood pressure has been shown to be more stable during normal pregnancy than in the nonpregnant state, 13, 14 presumably because venous tone is unchanged¹⁵ and the increase in blood volume occurring in normal pregnancy6 leads to less pronounced reductions of venous return during orthostatic provocations. A similarly improved tolerance to orthostatic stress has been noted in other conditions associated with increases in blood volume, such as heart failure.16 Our results confirm previous observations13.14 of a greater blood pressure stability in pregnancy, since systolic blood pressure was better maintained during tilting in the pregnant state in both groups. The tiltinginduced increases in arterial norepinephrine levels were also smaller, indicating a less pronounced increase in sympathetic nerve activity during orthostatic stress in pregnancy. It is interesting to note that the arterial plasma epinephrine levels failed to increase during tilting in the healthy pregnant women. This is a further indication that normal pregnancy is associated with increased cardiovascular stability during orthostatic stress, thereby requiring less activation of sympathoadrenal counterregulatory responses.

The increase in heart rate during tilting was less pronounced during pregnancy in both groups of subjects, in agreement with earlier findings in healthy pregnant women.13, 14 This may be related to a less intense activation of cardiac sympathetic nerve fibers and/or to a less pronounced vagal withdrawal during tilting in the pregnant state. Our finding that the arterial plasma norepinephrine levels increased less when tilting was performed during pregnancy in both groups suggests that a reduced sympathetic response to tilting was of importance. Regardless of the mechanism behind the blunted increase in heart rate, it is evident that cardiac

output is adequately maintained during orthostatic stress in the pregnant state, presumably because of less pronounced reductions of stroke volume secondary to the improvement of venous return. In agreement with this interpretation we found increases in blood volume during pregnancy in both groups, albeit blunted in the pregnancy-induced hypertension group. It should be noted that during pregnancy the subjects were studied in the left lateral position in order to reduce the influence of the pregnant uterus on venous return in the recumbent position.

The forearm vasoconstrictor response to tilting was completely abolished during normal pregnancy in our study. The absence of a vasoconstrictor response in the control group may well be explained by the increase in blood volume and consequently in venous return during pregnancy, as discussed above. In addition, it is possible that vasoconstrictor responses to sympathetic nerve activation are reduced during pregnancy, a mechanism supported by our findings in these patients in connection with somatomotor activation induced by a cold pressor test (see the forthcoming paper). The cardiovascular responses to norepinephrine in pregnancy are being investigated further in our laboratory.

During pregnancy the pregnancy-induced hypertension group had a significantly greater arterial norepinephrine response to tilting than the control group. The plasma epinephrine and forearm vasoconstrictor responses to tilting were abolished by normal pregnancy but were intact in the pregnancy-induced hypertension group. This may be related to an inadequate increase in blood volume in these patients during pregnancy, leading to cardiovascular volume receptor deactivation and reflex vasoconstriction in the forearm during tilting,1,12 as discussed above. In all patients blood pressures were maintained during tilting in the pregnant state, indicating that arterial baroreceptor mechanisms probably were of minor importance. Thus a predominant deactivation of volume receptors may explain why the pregnancy-induced hypertension group demonstrated an enhanced vasoconstrictor response but a similar heart rate response to tilting as compared with responses of the control group in the pregnant state.1.12 The hypothesis of a volume-dependent influence on sympathetic nerve activity in pregnant patients with pregnancy-induced hypertension is supported by our finding of inverse correlations between arterial norepinephrine concentrations and blood volume both at rest and during tilting. Such correlations were not found in the control group during pregnancy or in either group post partum.

In conclusion, the normal cardiovascular and sympathoadrenal adjustments to orthostatic stress were found to be blunted in normal pregnancy, presumably because of blood volume expansion leading to improved venous return to the heart in the upright position. Reduced sensitivity to vasoconstrictor stimuli may have contributed. In pregnancy-induced hypertension the pregnancy-induced increase in blood volume was less pronounced, which may explain the larger cardiovascular and sympathoadrenal responses of this group to tilting during pregnancy.

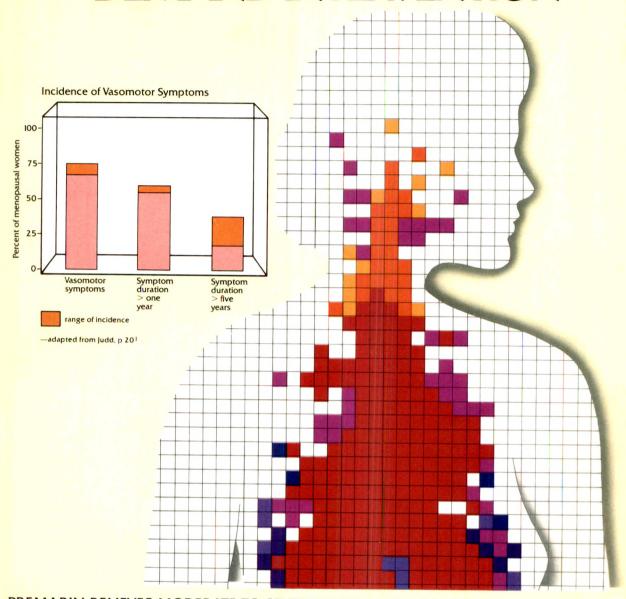
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VASOMOTOR SYMPTOMS THAT DEMAND INTERVENTION



PREMARIN RELIEVES MODERATE TO SEVERE VASOMOTOR SYMPTOMS

Vasomotor symptoms are the most common manifestation of the menopause, affecting up to 75% of menopausal women. Of these, 80% may suffer for more than a year and up to 50% for more than five years. These symptoms can disrupt a woman's life by chronically interrupting sleep, resulting in anxiety and irritability.

In a study of postmenopausal women suffering severe episodes of cutaneous flushing, symptoms improved markedly after administration of estrogen²—the treatment of choice for moderate to severe vasomotor symptoms. The estrogen of choice is PREMARIN, the most widely prescribed estrogen for over 40 years. PREMARIN (Conjugated Estrogens Tablets, U.S.P.) relieves moderate to severe vasomotor symptoms of the natural menopause, as well as the acute and often severe symptoms of surgical menopause.

(CONJUGATED NS TABLETS, U.S.P.)







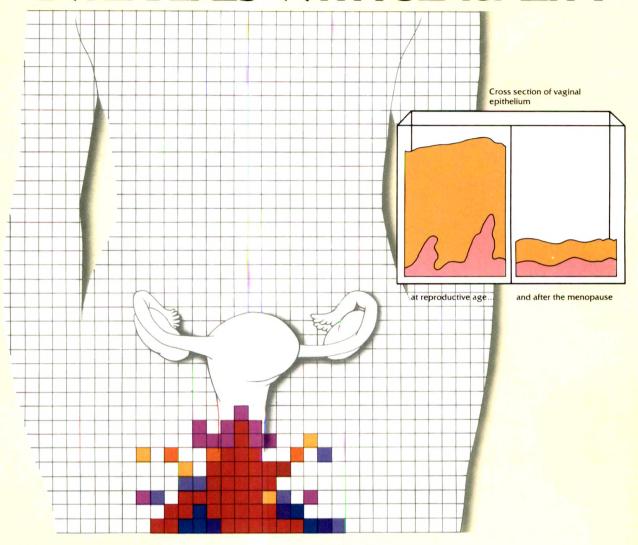




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VAGINAL ATROPHY THAT INTERFERES WITH SEXUALITY



PREMARIN RESTORES THE VAGINAL ENVIRONMENT

In the postmenopausal woman, decreasing levels of estrogen can have devastating effects on a woman's sexual functioning. The pH of vaginal secretions rises, promoting the growth of contaminating organisms. The vaginal epithelium dries and thins, becoming susceptible to irritation, injury, and infection. Sexual relations may be difficult or impossible.

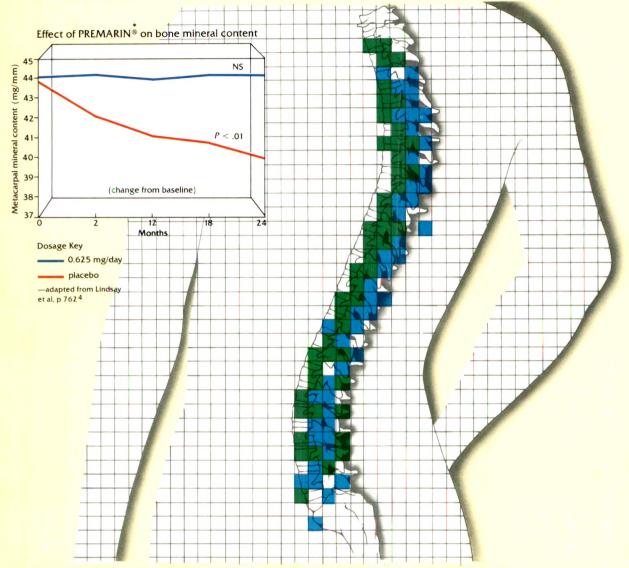
PREMARIN (Conjugated Estrogens, U.S.P.) Vaginal Cream focuses therapy at the site of the problem. Vaginal dryness is relieved, pH reverts to its normal acidity, and the epithelium thickens and becomes more resistant to injury and infection. With the vaginal environment returned to its premenopausal state, sexual function may improve.

PREMARIN® (CONJUGATED ESTROGENS, U.S.P.) Vaginal Cream



Please see last page for brief summary of full prescribing information.

POSTMENOPAUSAL BONE LOSS THAT INCAPACITATES



PREMARIN MAY HALT THE DISABLING COURSE OF OSTEOPOROSIS*

Osteoporosis has an enormous epidemiological impact: it affects 10 million American women, and 26% of all women over age 60.5 The disease begins silently and progresses inexorably for 15 to 20 years, until disabling complications occur.6

To minimize osteoporotic damage, the condition must be detected early and treated promptly. For many patients, PREMARIN is optimal therapy for osteoporosis, as part of a comprehensive program that includes exercise, good nutrition, and calcium supplements. In a controlled study of postmenopausal and oophorectomized women, PREMARIN (Conjugated Estrogens Tablets, U.S.P.) doses of 0.625 mg/day prevented loss of metacarpal mineral content (see graph above).⁴

PREMARIN® (CONJUGATED ESTROGENS TABLETS, U.S.P.)











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*Conjugated Estrogens Tablets have been evaluated as probably effective for estrogen-deficiency-induced osteoporosis.

Please see last page for brief summary of full prescribing information.

BRIEF SUMMARY (FOR FULL PRESCRIBING INFORMATION AND PATIENT INFORMATION, SEE

PREMARIN® Brand of Conjugated Estrogens Tablets, U.S.P.
PREMARIN® Brand of Conjugated Estrogens, U.S.P.
Vaginal Cream in a nonliquefying base

ESTROGENS HAVE BEEN REPORTED TO INCREASE THE RISK OF ENDOMETRIAL

CARCINOMA. Three independent case control studies have reported an increased risk of endometrial cancer in postmenopausal women exposed to exogenous estrogens for more than one year. This risk was independent of the other known risk factors for endometrial cancer. These-studies are further supported by the finding that incidence rates of endometrial cancer have increased sharply since 1969 in eight different areas of the United States with population-based cancer sharply since 1969 in eight different areas of the United States with population-based cancer reporting systems, an increase which may be related to the rapidly expanding use-of estrogens during the last decade. The three case control studies reported that the risk of-endometrial cancer in estrogen users was about 4.5 to 13.9 times greater than in nonusers. Themsk appears to depend on both duration of treatment and on estrogen dose. In view of these findings, when estrogens are used for the treatment of menopausal symptoms, the lowest dose that will control symptoms should be utilized and medication should be discontinued as soon-as possible. When prolonged treatment is medically indicated, the patient should be reassessed on at least a semiannual basis to determine the need for continued therapy. Although the evidence must be considered preliminary, one study suggests that cyclic administration of low doses of estrogen may carry less risk than continuous administration; if therefore appears prudent to utilize such a regimen. Close clinical surveillance of all women taking estrogens is important. In all cases of undiagnosed persistent or recurring abnormal vaginal bleeding, adequate diagnistic measures should be undertaken to rule out malignancy. There is no evidence at present that "natural" estrogens are more or less hazardous than "synthetic" estrogens at equiestrogenic doses.

doses.
2. ESTROGENS SHOULD NOT BE USED DURING PREGNANCY 2. ESTROGENS SHOULD NOT BE USED DURING PREGNANCY
The use of female sex hormones, both estrogens and progestogens, during early pregnancy
may seriously damage the offspring. It has been shown that females exposed in utero to
diethylstilbestrol, a non-steroidal estrogen, have an increased risk of developing in later life a
form of vaginal or cervical cancer that is ordinarily extremely rare. This risk has been selmated
as not greater than 4 per 1000 exposures. Furthermore, a high percentage of such exposed
women (from 30 to 90 percent) have been found to have vaginal adenosis, epitheliak-manges of
the vagina and cervix. Although these changes are histologically benign, it is not known whether
they are precursors of malignancy. Although similar data are not available with the use of other
estrogens, it cannot be presumed they would not induce similar changes. Several reports
suggest an association between intrauterine exposure to female sex hormones and congenital
anomalies, including congenital heart defects and limb reduction defects. One case control suggest an association between intrauterine exposure to female sex hormones and congenital heart defects and limb reduction defects. One case control study estimated a 4.7-fold increased risk of limb reduction defects in infants exposed mutero to sex hormones (oral contraceptives, hormone withdrawal tests for pregnancy, or attempted treatment for threatened abortion). Some of these exposures were very short and invalved only a few days of treatment. The data suggest that the risk of limb reduction defects in exposed during pregnancy in an attempt to treat threatened or habitual abortion. There is comsiderable evidence that estrogens are ineffective for these indications, and there is no evidence from well controlled studies that progestogens are effective for these uses. If PREMARIN is usuad during pregnancy, or if the patient becomes pregnant while taking this drug, she should be apprised of the potential risks to the fetus, and the advisability of pregnancy continuation.

DESCRIPTION: PREMARIN (Conjugated Estrogens, U.S.P.) contains a mixture of estrogens, obtained exclusively from natural sources, blended to represent the average composition of material derived from pregnant mares' urine. It contains estrone, equilin, and 17α -dihydroequim, logether with smaller amounts of 17α -estradiol, equilenin, and 17α -dihydroequilenin as salts of their sulfate

INDICATIONS: Based on a review of PREMARIN Tablets by the National Academy of Sciences—National Research Council and/or other information, FDA has classified the

Sciences – National Research Council and/or other information, FDA has classified the indications for use as follows:

Effective: 1. Moderate to severe vasomotor symptoms associated with the menopause. (There is no evidence that estrogens are effective for nervous symptoms or depression without associated vasomotor symptoms, and they should not be used to treat such conditions.)

2. Atrophic vaginitis

3. Kraurosis vulvae

4. Except by progregations.

- Female hypogonadism
- Female castration Primary ovarian failure
- Breast cancer (for palliation only) in appropriately selected women and men with static disease
- metastatic disease.

 8. Prostatic carcinoma palliative therapy of advanced disease.

 9. Postpartum breast engorgement Although estrogens have been widely used for the prevention of postpartum breast engorgement, controlled studies have demonstrated hat the incidence of significant painful engorgement in patients not receiving such hormonal therapy is low and usually responsive to appropriate analgesic or other supportive therapy. Consequently, the benefit to be derived from estrogen therapy for this indication must be carefully weighed against the potential increased risk of puerperal thromboembolism associated with the use of large doses of estrogens.

 PREMARIN HAS NOT BEEN SHOWN TO BE EFFECTIVE FOR ANY PURPOSE DURING PREGNANCY AND ITS USE MAY CAUSE SEVERE HARM TO THE FETUS (SEE BOXED WARNING).

WARNING).
"Probably" effective: For estrogen deficiency-induced osteoporosis, and only when used in conjunction with other important therapeutic measures such as diet, calcium, physiotherapy, and good general health-promoting measures. Final classification of this indication requires further investigation.

INDICATIONS: PREMARIN (Conjugated Estrogens, U.S.P.) Vaginal Cream is indicated in the treatment of atrophic vaginitis and kraurosis vulvae. PREMARIN Vaginal Cream HAS NOT BEEN SHOWN TO BEFFECTIVE FOR ANY PURPOSE DURINING PREGNANCY AND ITS USE MAY CAUSE SEVERE HARM TO THE FETTUS (SEE BOXED WARNING).

CONTRAINDICATIONS: Estrogens should not be used in women (or men) with any of the following conditions: I. Known or suspected cancer of the breast except in appropriately selected patients being treated for metastatic disease. 2. Known or suspected estrogen-dependent metaplasia. 3. Known or suspected pregnancy (See Boxed Warning). 4. Undiagnosed abnormal genitalbleeding. 5. Active thrombophiebitis or thromboembolic disorders. 6. A past history of thrombophiebitis, thrombosis, or thromboembolic disorders associated with previous estrogen use (except when used in treatment of breast or prostatic malignancy).

5. Active thrombophlebitis or thromboembolic disorders. 6. A past history of thrombophlebitis, hormbosis, or thromboembolic disorders associated with previous estrogen use (except when used in treatment of breast or prostatic malignancy).
WARNINGS: Long term continuous administration of natural and synthetic estrogens in eertain animal species increases the frequency of carcinomas of the breast, cervix, vagina, and liver. There are now reports that estrogens increase the risk of carcinoma of the endometrium in humans. (See Boxed Warning.) At the present time there is no satisfactory evidence that estrogens. Given to postmenopausal women increase the risk of cancer of the breast, although a recent study has raised this possibility. There is a need for caution in prescribing estrogens for women with a strong family history of breast cancer or who have breast nodules, fibrocystic disease, or abnormal mammograms. A recent study has reported a 2- to 3-fold increase in the risk of surgically confirmed gatibloider disease in women receiving postmenopausal estrogens.
Adverse effects of oral contraceptives may be expected at the larger doses of estrogen used to treat prostatic or breast cancer or postpartum breast engorgement; it has been shown that there is an increased risk of thrombosis in men receiving estrogens for prostatic cancer and women for postpartum breast engorgement. Users of oral contraceptives have an increased risk of thrombosis, and optic neuritis have been reported in oral contraceptive users. An increased risk of postsurgery thromboembolic complications has also been reported in users of oral contraceptives. If feasible, estrogen should be discontinued at least 4 weeks before surgery of the type associated with an increased risk of thromboembolism, or during periods of prolonged immobilization. Estrogens should not be used in persons with active thrombombolistis, thromboembolic disorders, or in persons with a history of such disorders in association with estrogen use. They should be Large doses (5 mg conjugated estrogens per day), comparable to those used to treat cancer of the prostate and breast have been shown to increase the risk of nonfatal myocardial interction,

pulmonary embolism and thrombophlebitis. When doses of this size are used, any of the

pulmonary embolism and thrombophlebitis. When doses of this size are used, any of the thromboembolic and thrombotic adverse effects should be considered a clear risk.

Benign hepatic adenomas should be considered in estrogen users having abdominal pain and tenderness, abdominal mass, or hypovolemic shock. Hepatocellular carcinoma has been reported in women taking estrogen-containing oral contraceptives. Increased blood pressure may occur with use of estrogens in the menopause and blood pressure should be monitored with estrogen use. A worsening of glucose tolerance has been observed in patients on estrogen-containing oral contraceptives. For this reason, diabetic patients should be carefully observed. Estrogens may lead to severe hypercalcemia in patients with breast cancer and bone metastases.

PRECAUTIONS: Physical examination and a complete medical and family history should be taken prior to the initiation of any estrogen therapy with special reference to blood pressure, breasts, abdomen, and pelvic organs, and should include a Papanicolau smear. As a general rule, estrogen should not be prescribed for longer than one year without another physical examination being performed. Conditions influenced by fluid retention such as asthma, epilepsy, migraine, and cardiac or renal dysfunction, require careful observation. Certain patients may develop manifestations of excessive estrogenic stimulation, such as abnormal or excessive uterine bleeding, mastodynia, etc. Prolonged administration of unopposed estrogen therapy has been reported to increase the risk of endometrial hyperplasia in some patients. Oral contraceptives appear to be associated with an increased incidence of mental depression. Patients with a history of depressions should be carefully observed. Preexisting uterine leiomyomata may increase in size during estrogen use. The pathologist should be advised of estrogen therapy when relevant specimens are submitted. If jaundice develops in any patient receiving estrogen, the medication should be discontinued

b Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability.
c. Increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by PBI, T4 by column, or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG; free T4 concentration is unaltered.
d. Impaired glucose tolerance.
e. Decreased pregnanediol excretion.
f. Reduced response to metyrapone test.
g. Reduced serum folate concentration.

f. Reduced response to metyrapone test.
g. Reduced serum friglyceride and phospholipid concentration.
h. Increased serum triglyceride and phospholipid concentration.
As a general principle, the administration of any drug to nursing mothers should be done only when clearly necessary since many drugs are excreted in human milk.

ADVERSE REACTIONS: The following have been reported with estrogenic therapy, including oral contraceptives: breakthrough bleeding, spotting, change in menstrual flow, dysmenorrhea; premenstrual-like syndrome; amenorrhea during and after treatment; increase in size of uterine fibromyemata; vaginal candiciasis, change in cervical erosion and in degree of cervical escribin crystitis-like syndrome; tenderness, enlargement, secretion (of breasts); nausea, vomiting, abdominal cramps, bloating; cholestatic jaundice; chloasma or melasma which may persist when drug is discontinued; erythema multiforme; erythema nodosum; hemorrhagic eruption; loss of scalp hair; hirsutism; steepening of corneal curvature; intolerance to contact lenses; headache, migraine, dizziness, mental depression, chorea; increase or decrease in weight; reduced carbohydrate tolerance; aggravation of porphyria; edema; changes in libido.

ACUTE OVERDOSAGE: May cause nausea, and withdrawal bleeding may occur in females.

DOSAGE AND ADMINISTRATION:

PREMARIN® Brand of Conjugated Estrogens Tablets, U.S.P.

1. Given eyclically for short-term use only. For treatment of moderate to severe vasomotor symptoms, atrophic vaginitis, or kraurosis vulvae associated with the menopause (0.3 to 1.25 mg or more daily).

The lowest dose that will control symptoms should be chosen and medication should be discontinued as promptly as possible.

Administration should be cyclic for three weeks on and one week off).

discontinued as promptly as possible.

Administration should be cyclic (e.g., three weeks on and one week off).

Attempts to discontinue or taper medication should be made at three to six month intervals.

2. Given cyclically: Female hypogonadism. Female castration. Primary ovarian failure. Osteoporo-

SIS. Female hypogonadism—2.5 to 7.5 mg daily, in divided doses for 20 days, followed by a rest period of 10 days' duration. If bleeding does not occur by the end of this period, the same dosage schedule is repeated. The number of courses of estrogen therapy necessary to produce bleeding may vary depending on the responsiveness of the endometrium.

If bleeding occurs before the end of the 10 day period, begin a 20 day estrogen-progestin cyclic if bleeding occurs before the end of the 10 day period, begin a 20 day estrogen-progestin cyclic regimen with PREMARIN (Conjugated Estrogens Tablets, U.S.P.), 2.5 to 75 mg daily in divided dose for 20 days. During the last five days of estrogen therapy, give an oral progestin. If bleeding occurs before this regimen is concluded, therapy is discontinued and may be resumed on the fifth day of

bleeding.

Female castration and primary ovarian failure—1.25 mg daily, cyclically. Adjust upward or downward according to response of the patient. For maintenance, adjust dosage to lowest level that will provide effective control.

will provide effective control.

Osteoperosis (it) retard progression) — 1.25 mg daily, cyclically.

3. Given for a few days. Prevention of postpartum breast engorgement — 3.75 mg every four hours for five doses, or 1.25 mg every four hours for five days.

4. Given chronically. Inoperable progressing prostatic cancer — 1.25 to 2.5 mg three times daily. Inoperable progressing breast cancer in appropriately selected men and postmenopausal women — 10 mg three times daily for a period of at least three months.

Patients with an intact uterus should be monitored for signs of endometrial cancer and appropriate measures taken to rule out malignancy in the event of persistent or recurring abnormal vaginal bleacting.

bleeding

PREMARIN* Brand of Conjugated Estrogens, U.S.P. Vaginal Cream

Given cyclically for short-term use only. For treatment of atrophic vaginitis or kraurosis vulvae.

The lowest dose that will control symptoms should be chosen and medication should be discontinued as promptly as possible.

Administration should be cyclic (e.g., three weeks on and one week off).

Attempts to discontinue or taper medication should be made at three to six month intervals.

Usual desage range: 2 to 4 g daily, intravaginally or topically, depending on the severity of the condition

Treated patients with an intact uterus should be monitored closely for signs of endometrial cancer and appropriate diagnostic measures should be taken to rule out malignancy in the event of persistent

and appropriate diagnostic measures should be taken to rule out malignancy in the event of persistent or recurring abnormal vaginal bleeding. **HOW SUPPLIED:** PREMARIN (Conjugated Estrogens Tablets, U.S.P.). No. 865 — Each *purple* tablet contains 2.5 mg in bottles of 100 and 1,000. No. 866 — Each *yellow* tablet contains 1.25 mg in bottles of 100 and 1,000. Also in Cycle Pack of 21 and in unit dose package of 100. No. 864 — Each *witle* tablet contains 0.9 mg in bottles of 100. Also in Cycle Pack of 21. No. 867 — Each *maroon* tablet contains 0.625 mg in bottles of 100. Also in Cycle Pack of 21 and unit dose package of 100. No. 868 — Each *green* tablet contains 0.3 mg in bottles of 100 and 1,000. The appearance of these tablets is a trademark of Ayerst Laboratories.

PEPMARIN Conjugated Estrogens** LLS P.J. Varinal Cream — No. 872 — Each gray contains.

trademark of Ayerst Laboratories PREMARIN (Conjugated Estrogens, U.S.P.) Vaginal Cream – No. 872 – Each gram contains 0.625 mg Conjugated Estrogens, U.S.P. (Also contains cetyl esters wax, cetyl alcohol, white wax, glyceryl monostearate, propylene glycol monostearate, methyl stearate, phenylethyl alcohol, sodium lauryl sulfate, glycerin, and mineral oil.) Combination package: Each contains Net Wt. 11/2 oz. (42.5 g) tube with one calibrated plastic

applicator.

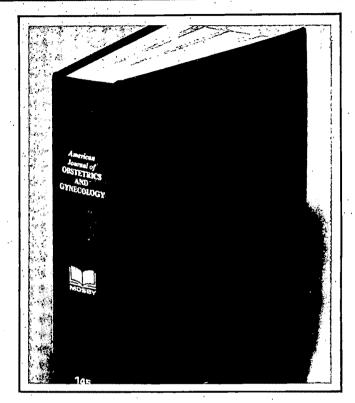
Also Available – Refill package: Each contains Net Wt. 1½ oz. (42.5 g) tube.

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ABS-044

A Near-Spotless Record.

Other OCs claim it. We show it.1*

In clinical trials, Lo/Ovral was practically perfect in holding spotting and breakthrough bleeding to a minimum.

	SPOTTING	Breakthrough Bleeding
Cycle 1	10.6%	8.8%
Cycle 3	6.3%	3.5%
Total Cycles‡	4.2%	2.9%

Lo/Ovral® offers less breakthrough bleeding and spotting, along with high contraceptive efficacy, at a low dose—just 30 mcg ethinyl estradiol and 0.3 mg norgestrel.

LOW DOSE

Dickey RP: Managing Contraceptive Pill Patients, 3rd ed., Durant, Oklahoma, Creative Infomatics, Inc., 1983.

Rates are derived from separate studies from different investigators in several population groups and cannot be compared precisely.

Serious as well as minor adverse reactions have been reported following the Lse of all oral contraceptives.

confracapitives.

‡Data on file for 22,489 total cycles, Wyeth Laboratories. See important information on following page.

IN BRIEF

Indications and Usage—LO/OVRAL® is indicated for the prevention of pregnancy in wor en who elect to use oral contraceptives (OCs) as a method of contraception.

use oral contraceptives (OC's) as a method of contraception.

Contraindications—OC's should not be used in women with any of the following condition:

1. Thrombophlebitis or thromboembolic disorders.

2. A past history of deep-vein thrombophlebitis or thromboembolic disorders.

3. Gerebra-lvascular or coror ary-artery disease.

4. Known or suspected carcinoma of the breast.

5. Known or suspected estrogen-dependent neoplasia.

6. Undiagnosed abnormal genital bleeding.

7. Known or suspected pregnancy (see Warning No. 5).

8. Benign or malignant liver tumor which developed during use of OC's or other estroge montaining products.

Warnings

Cigarette smoking increases the risk of serious cardiovascular side effects from oral =ort_aceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day ab is quite marked in women over 35 years of age. Women who use oral contraceptives should be string_ advised not to smoke.

not to shoke.

The use of oral contraceptives is associated with increased risk of several serious conciticns, including thromboembolism, stroke, myccardial infarction, hepatic adenoma, galibladder disease, yeatension. Practitioners prescribing oral contraceptives should be familiar with the following information — lating to these disks.

1. Thromboembolic Disorders and Other Vascular Problems—An increased risk of thrombe.r⊃olic and thrombotic disease associated with use of OC's is well established. Three principal studies is G ⇒t Britain and 3 in the U.S. have demonstrated increased risk of fatal and nonfatal venous thrombo manism and stroke, both hemorrhagic and thrombotic. These studies estimate that users of OC's are 4 to 1 incs more likely than nonusers to develop these diseases without evident cause. CEREBROVASCULAR DISORDERS—In a collaborative American study of cerebrovascula d ⊕orders in women with and without predisposing causes, it was estimated that the risk of hemorrhagic store was 2.0 times greater in users than nonusers and the risk of thrombotic stroke was 4 to 9.5 times greate in users than in nonusers.

CREBROVASCULAR DISORDERS—In a collaborative American study of cerebrovascula d ⊕rders in women with and without predisposing causes, it was estimated that the risk of hemorrhagic error- was 2.0 times greater in users than nonusers and the risk of thrombotic stroke was 4 to 9.5 times greater in users than in nonusers.

MYOCARDIAL INFARCTION (MI)—An increased risk of MI associated with use of OC's has been eported, confirming a previously suspected association. These studies, conducted in the UK, found, as expected, confirming a previously suspected association. These studies, conducted in the UK, found, as expected, that the greater the number of underlying risk factors for coronary artery disease (cigarette moking, hypertension, hypercholesterolemia, obesity, diabetes, history of pre-ectamptic toxemia) the right the risk of developing MI, regardless of whether the patient was an OC user on rol. OC's, however, were ficand to be a clear additional risk factor. In terms of relative risk, it has been estimated that OC users who "o a 1 smoke smoking is considered a major predisposing condition to MII] are about those as likely to have a Eral MI as nonusers who do not smoke. Oc users who are also smokers have about a 5-fold increased risk. * Istal MI compared to users who do not smoke, but about a 10-to 12-fold increased risk compared to rome ers who do not smoke but about a 10-to 12-fold increased risk compared to rome ers who do not smoke use to the service of the rest of various age groups must be given service. * Istal MI compared to rome very the production of these relative risk, in the service of the rest of various age groups must be given services. * Istal MI compared to rome very the production of the service of the rest of various and the production of the service of the rest of various must be given services. * Istal MI compared to rome years of the compared to rome years and the production of the

increased risk of MI persisted for at least 9 years in women 40 to 49 years old who had used \$\tilde{C}^*s\$ or 5-or more years. Findings in both studies require confirmation since they are inconsistent with chembublished information.

2. Ocular Lesions—There have been reports of neuro-ocular lesions such as optic neuritis or minor thrombosis associated with use of OC's. Discontinue OC's if there is unexplained, sudden or gradual, artital or complete loss of vision; onset of proptosis or diplopia; papilledema: or ret nal-vascular (espat, and institute appropriate diagnostic and therapeutic measures.

3. Carcinoma—Long-term continuous administration of either natural or synthetic estrogen in the continuous administration of either natural or synthetic estrogen in the corresponding of the breast, cervix, vagina, and liver. Certain synthetic progestogens, none currently contained in OC's, have been noted to increase incidence of man harmonisms of the breast, cervix, vagina, and liver. Certain synthetic progestogens, none currently contained in OC's, have been noted to increase incidence of man harmonisms of endometrial carcinoma associated with prolonged use of exogenous estrogen in postme objects of endometrial carcinoma associated with prolonged use of exogenous estrogen in postme objects of adenocarcinoma of the endometrium in women under 40 on OC's. Of cases found in women who but predisposing risk factors (e.g., irregular) beeding at the time OC's were first given, polycystic ovariety, really all occurred in women who had used a sequential OC. These are no longer marketed. No evidence shall occurred in women who had used a sequential occurred in women have have a sequential occurred in women having OC=3 or estrogens. One study, however, while also noting no overall increased risk of concern marketed. No evidence from human studies have found no increase in breast cancer in women having OC=3 or estrogens. One study, however, while also noting no overall increased risk of breast cancer in women having OC=3 or es

@ 1984, Wyeth Laboratories.

malignancy. Male children so exposed may develop abnormalities of the urogenital tract. Although similar data are not available with use of other estrogens, it cannot be presumed they would not induce similar changes. An increased risk of congenital anomalies, including heart defects and limb defects, has been reported with use of sex hormones, including OC's, in pregnancy. One case control study estimated a 4.7-fold increase in risk of limb-reduction defects in infants exposed in utero to sex hormones (OC's, hormonal withdrawal tests for pregnancy, or attempted treatment for threatened abortion). Some exposures involved only a few days. Data suggest that risk of limb-reduction defects in exposed feluses is some exposures involved only a few days. Data suggest that risk of limb-reduction defects in exposed feluses is somewhat less than 1 in 1,000 live births. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective to these indications, and there is no evidence from well-controlled studies that progestogens are effective. There is some evidence that triploidy and possibly other types of polyploidy are increased among abortuses from women who become pregnant soon after ceasing OC's. Embryos with these anomalies are virtually always aborted spontaneously. Whether there is an overall increase in spontaneous abortion of pregnances conceived soon after stopping OC's is unknown. It is recommended that, for any patient who has missed 2 consecutive periods, pregnancy should be ruled out before continuing OC's. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at time of first insed period, and further use of OC's should be withheld until pregnancy has been ruled out. If pregnancy is confirmed, the patient should be apprised of the potential risks to the fetus, and advisability of continuation of the pregnancy should be discussed. It is a

pareinty observed while of O.C.s. Increase in triglycendes and total prospholipids has been doserved materials no CC's; clinical significance of this finding remains to be defined.

8. Elevated Blood Pressure—Increase in blood pressure has been reported in patients on OC's. In some women, hypertension may occur within a few months of beginning OC's. In the 1st year of use, prevalence of women with hypertension is low in users and may be no higher than that of a comparable group of nonusers. Prevalence in users increases, however, with longer exposure, and in the 5th year of use is 2½ be 3 times the reported prevalence in the 1st year. Age is also strongly correlated with development of hypertension in OC users. Women who previously have had hypertension during pregnancy may be more likely to develop elevation of blood pressure on OC's. Hypertension that develops as a result of taking OC's usually returns to normal after discontinuing the drug.

S. Headanch—Onset or exacerbation of migraine or development of headache of a new pattern which is recurrent, persistent, or severe, requires discontinuation of OC's and evaluation of the cause.

recurrent, persistent, or severe, requires discontinuation of OCs and evaluation of the cause.

13. Bleeding Irregularities—Breakthrough bleeding, spotting, and amenorrhea are frequent reasons for patients discontinuing OCs. In breakthrough bleeding, as in all cases of irregular vaginal bleeding, nonfunctional causes should be borne in mind.

In undiagnosed persistent or recurrent abnormal bleeding from the vagina, adequate diagnostic measures are indicated to rule out pregnancy or maignancy. If pathology has been excluded, time or change to another OC may solve the problem. Changing to an OC with a higher estrogen content, while potentially useful in minimizing menstrual irregularity, should be done only if necessary, since this may increase risk more more than the problem change of the problem change

without previous irregularities.

11. Eclopic Pregnancy—Ectopic as well as intrauterine pregnancy may occur in contraceptive failures.

12. Breast-feeding—OC's given in the postpa-tum period may interfere with lactation and decrease quantity and quality of breast milk. Furthermore, a small fraction of the hormones in OC's has been identified in the milk of mothers on OC's, effects, if an,, on the breast-fed child have not been determined. If feasible, defer OC's until infant has been weared.

Precautions—GENERAL—I. A complete medical and family history should be taken prior to initiation of OC's. Pretratment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Pap smear and relevant laboratory tests.

As a general rule OC's should not be prescribed for longer than 1 year without another physical examination.

2. Under influence of estrogen-progestogen preparations, pre-existing uterine leiomyomata may increase

in size.

3. Patients with history of psychic depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. Patients becoming significantly depressed while on OC's should stop OC's and use an alternate method to try to determine whether the symptom is drug-related.

4. OC's may cause some degree of fluid reter fron. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention, such as convolvable disorders, migratine syndrome, asthma, or cardiac or renal insufficiency.

5. Patients with a past history of jaund ce ouring pregnancy have an increased risk of recurrence while on OC's. If jaundice develops, OC's should be discontinued.

6. Sterrid homopous may be propriy metabolized in patients with impagied liver function and should be

6. Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution.

CC users may have disturbances in normal tryptophan metabolism which may result in a relative pyridoxine deficiency. Clinical significance is undetermined.

7. OC users may have disturbances in normal tryptophan metabolism which may result in a relative pyridoxine deficiency. Clinical significance is undetermined.
8. Sarum folate levels may be depressed by OC's. Since the pregnant woman is predisposed to development of folate deficiency and incidence of folate deficiency increases with increasing gestation, it is possible that if a woman becomes pregnant shortly after stopping OC's, she may have a greater chance of developing folate deficiency and complications attributed to this deficiency.
9. The pathologist should be advised of OC therapy when relevant specimens are submitted.
10. Certain endocrine- and liver-function tests and blood components may be affected by estrogen-containing OC's: a. Increased sulfobromophitalein retention. b. Increased prothombin and factors VII, VIII, IX, and X. decreased attribumbin 3; increased norpinephrine-induced platelet aggregability. C. Increased thyrcid-binding globulin (TBG) leading to increased circulating total-thyroid hormone, as measured by protein-bound iodine (PBI). The by column, or 14 by radiommunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG; free T4 concentration is unaltered.
d. Decreased pregnanediol excretion.
e. Reduced response to metyrapone test.
Information for the Patient—See Patient Package Labeling.
Drug Interactions—Reduced efficacy and increased incidence of breakthrough bleeding have been associated with concomitant use of rifampn. A similar association has been suggested with barbiturates, phenyloulazone, phenytoin sodium, ampicillin and letracycline.
Carcinogenesis—See Warnings on carcinogenic potential of OC's.
Pregnancy—Category X. See Contraindications, Warnings.
Nursing Mothers—See Warnings on carcinogenic potential of OC's.
Pregnancy—Category X. See Contraindications, Warnings.
Nursing Mothers—See Warnings on association between the following conditions and use of OC's athord additional

optic neuritis.

The following adverse reactions have been reported in patients on OC's and are believed to be drug-related. Nausca and/or vomiting, usually the most common adverse reactions, occur in approximately 10 percent or less of patients during the first cycle. Other reactions, as a general rule, are seen much less frequently or only occasionally, Gastrointestinal symptoms isuch as abdominal cramps and bloating), breakthrough bleeding, spotting, change in menstrual flow; dysmenorrhea, amenorrhea during and after treatment, temporary infertility after discontinuance of treatment, edema; chloasma or melasma which may persib reast changes: tenderness, enlargement and secretion; change in weight (increase or decrease), change in cervical erosion and cervical secretion; possible diminution in lactation when given immediately postpartum; cholestatic jaundice; migraine; increase in size of uterine leiomyomata; rash (allergic); mental depression; reduced tolerance to carbohydrates vaginal candidiasis; change in corneal curvature (steepening), intolerance to carbohydrates vaginal candidiasis; change in corneal curvature (steepening).

Intolerance to contact lenses.

The following adverse reactions have been reported in users of OC's, and the association has been neither confirmed nor refuled: premenstrual-like syndrome; cataracts, changes in libido, chorea, changes in appetite, cystitis-like syndrome, headache, nervousness, dizziness, hirsutism, loss of scalp hair, erythema multiforme, erythema nodosum, hemorrhagic eruption, vaginitis, porphyria.

Acute Overdose—Serious ill effects have not been reported following acute ingestion of large doses of OC's by young children. Overdosage may cause nausea, and withdrawal bleeding may occur in females.





Zinc and α -fetoprotein in amniotic fluid from early pregnancies with fetal malformations

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Zinc and α-fetoprotein concentrations were quantitated in second-trimester amniotic fluid from 111 normal pregnancies and 29 pregnancies with various fetal malformations. The zinc level varied between 0.3 and 4.0 μmol/L (median 1.3) in the normal samples and between 1.8 and 17.9 μmol/L (median 5.0) in cases with malformations. The level was above 2.5 times the normal median in 23 of the 29 (79%) gestations with the malformations. Amniotic fluid zinc and α -fetoprotein levels showed a cositive correlation (r = 0.78), and the α -fetoprotein level was elevated in all gestations with fetal disorders and elevated zinc levels, as well as in five of the six cases with fetal defects but normal zinc concentrations. The elevation of zinc was more marked than that of α-fetoprotein in three cases of fetal malformations. Five samples were false positive on α -fetoprotein assay but contained normal zinc levels. Removal of α -fetoprotein from amniotic fluid had no effect on the zinc concentration, indicating that zinc is not bound to amniotic fluid αfetoprotein. (Am J OBSTET GYNECOL 1985;152:561-5.)

Key words: Prenatal diagnosis, congenital malformations, early amniotic fluid, zinc, α-fetoprotein

Human amniotic fluid contains constant levels of zinc (1 to 3 μmol/L)1-2a until the thirty-seventh week of gestation, when a sharp increase in the mean concentration occurs.3 Recently, Parkinson et al.4 studied zinc and copper concentrations in early amniotic fluid from pregnancies with neural tube defects and found elevated zinc levels in cases with open lesions. They also observed a highly significant correlation (r = 0.83) between zinc and α-fetoprotein levels, suggesting that zinc might be bound to α-fetoprotein in amniotic fluid.4 α-Fetoprotein is known to bind copper,⁵ and recent studies have shown binding of zinc to α -fetoprotein in vitro.6

In this work, we have determined the zinc levels in amniotic fluid from second-trimester pregnancies with various fetal malformations and studied the relationship between amniotic fluid zinc and α-fetoprotein concentrations. The results indicate that a majority of pregnancies with fetal defects and elevated amniotic fluid α-fetoprotein concentrations also show elevated zinc levels. Removal of α-fetoprotein from amniotic fluid, however, had no effect on amniotic fluid zinc levels, indicating that zinc is not bound to α -fetoprotein.

Material and methods

Amniotic fluid samples. Zinc levels were measured in 140 amniotic fluid samples collected by amniocentesis for routine α-fetoprotein determinations and chromosomal analyses. Eighty-two samples were taken into normal plastic tubes and, after the α-fetoprotein determination, were stored at -20° C from 1 to 20 months before the zinc assay. The samples were centrifuged at 2000 rpm before the α-fetoprotein assay. Twenty-nine of these samples came from pregnancies with various fetal malformations, as listed in Table I, while 53 specimens were from pregnancies that ended in a delivery of a healthy child. The latter samples were selected so that the storage times in these two groups were similar.

The control material also included 58 samples from pregnancies with normal outcome, in which the zinc level was assayed soon after amniocentesis. These samples were aliquots of the routine AFP samples and were taken into acid-washed plastic tubes to avoid any zinc contamination, as described in paper cited in the Introduction. The zinc values in these samples (median 1.2 µmol/L, range 0.5 to 4.0) and in the control samples described above (median 1.3 µmol/L, range 0.3 to 3.0) did not differ from each other. Since the groups were also clinically similar, they were pooled into one control group (Fig. 1).

All samples came from singleton pregnancies. The

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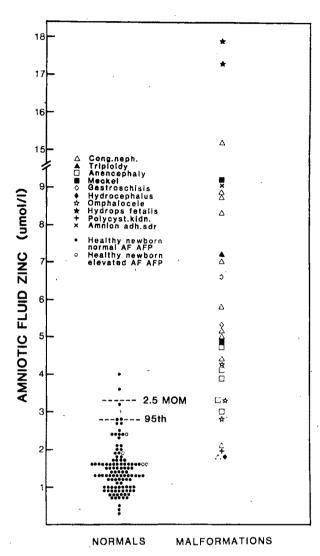


Fig. 1. Zinc levels in amniotic fluid at 15 to 20 weeks of gestation. *Normals*, Pregnancies that ended in a delivery of a healthy child.

indication for amniocentesis in most cases was a high maternal age (above 38 years) without any clinical evidence of fetal or maternal disorders. The gestational age in each case was determined by ultrasound scanning before the sample collection.

Assays. Zinc concentration was determined with atomic absorption spectrophotometry, as described in the paper cited above.

AFP was measured by double-antibody immunoassay at the Department of Bacteriology and Immunology, University of Helsinki. The samples were first centrifuged at 800 rpm for 10 minutes and then at 2000 rpm for 15 minutes before the α -fetoprotein measurement.

The zinc values in control samples had a skewed distribution and the elevations of zinc and α -fetoprotein levels are expressed as multiples of the median. The

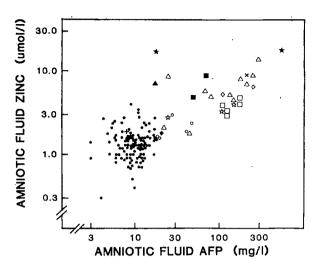


Fig. 2. Correlation of amniotic fluid zinc and α -fetoprotein levels in second-trimester pregnancies (r = 0.78). Symbols as in Fig. 1.

95 and 97.5 percentiles were 2.15 and 2.5 times the normal median in the zinc assay and 1.6 and 1.79 times the median in the α -fetoprotein assay.

Affinity chromatography. Anti-α-fetoprotein immunoadsorbent was prepared according to the method of Ruoslahti.8 The γ-globulin fraction from hyperimmune sheep anti-α-fetoprotein serum was isolated by sodium sulfate precipitation and ion-exchange chromatography on diethylaminoethyl Sephacel (Pharmacia Fine Chemicals, Uppsala, Sweden) and then passed through a Sepharose 4B column (Pharmacia) coupled with normal human serum proteins to abolish any reactivity with human albumin.9 The anti-α-fetoprotein globulin was further passed through a column of Blue-Sepharose (Pharmacia) to remove any contaminating albumin, which is the major zinc-binding protein in serum. Coupling of the antibodies to Sepharose 4B was performed according to the method of Axen et al.10 Normal sheep y-globulin adsorbent was prepared as the anti-α-fetoprotein Sepharose.

The binding capacity of the anti- α -fetoprotein Sepharose was approximately 67 μg of α -fetoprotein per 1 ml of adsorbent. Removal of α -fetoprotein from amniotic fluid was performed either by incubating 0 to 300 μl of anti- α -fetoprotein Sepharose with 1 ml of fresh amniotic fluid at room temperature for 2 hours (see Fig. 3) or by passing amniotic fluid samples (0.1 to 0.5 ml) through an anti-AFP column (bed volume 1 ml) (see Table III).

Results

The zinc concentrations in normal amniotic fluid varied between 0.3 and 4.0 µmol/L (median 1.3). The lev-

Table I. Amniotic fluid α-fetoprotein and zinc levels in second-trimester pregnancies

	α-fetoprotein				Zinc		
	No.	<2.5 MOM	2.5-5 MOM	>5 MOM	<2.5 MOM	2.5-5 MOM	>5 MOM
Normal	111	106	3	2	109	2	-
Congenital nephrosis	11		2	9	2	3	6
Triploidy	1		1	***************************************		***************************************	1
Anencephaly	5			5	1	4	
Meckel's syndrome	2			2		I	. 1
Gastroschisis	2			2		1	1
Omphalocele	3		1	2	1	2	
Hydrops fetalis	2		1	1			2
Hydrocephalus	1		1		1		
Polycystic kidneys	1	1			1		
Amnion adhesion syndrome	1	-	-	1		***************************************	1
Total	140	107	$\overline{9}$	24	115	13	12

No., Total number of samples; MOM, multiples of median.

Table II. Amniotic fluid α -fetoprotein and zinc levels in normal pregnancies with a false positive α-fetoprotein value and in gestations with malformations and moderately elevated α-fetoprotein level

Diagnosis	Week	Zirc		α-fetoprotein		
	of gestation	Concentration (µmol/L)	МОМ	Concentration (mg/L)	МОМ	
Normal	17	1.6	1.2	19,625	2.8	
Normal	18	1.6	1.2	19,750	3.0	
Normal	18	1.9	1.5	41,500	6.3	
Normal .	18	2.4	1.8	47,500	7.1	
Normal	15	3.0	2.3	28,000	2.9	
Congenital nephrosis	19	2.1	1.6	22,000	4.2	
Congenital nephrosis	16	8.7	6.7	25,000	3.1	
Triploidy	17	7.2	5.5	17,500	2.5	
Omphalocele	15	2.8	2.2	24,850	2.6	
Hydrops fetalis	20	17.3	13.3	17,500	4.3	
Hydrocephalus	17	1.8	1.4	20,835	3.0	
Pólycystic kidney	19	1.9	1.5	7,950	1.5	

MOM = Multiples of median.

Table III. Effect of removal of α -fetoprotein on amniotic fluid zinc levels in various types of samples

		Zinc	α-fetoprotein		
Diagnosis	Original concentration (µmol/L)	a-AFP/NsHS-S* (♀)	Original concentration (mg/L)	a-AFP/NShS-S* (%)	
Normal (n = 5)	0.9-1.7	96-104	4.1-13.3	<1-14	
Hydrops fetalis	17.9	35	540	40	
Omphalocele	4.3	117	161	<1	
Congenital nephrosis	9.1	97	250	6	
Congenital nephrosis	15.2	100	271	<1	
Anencephaly 1	3.3	130	125	<1	
Anencephaly	4.8	94	165	<1	
Pool (fetal disorders)†	3.4	120	75	5	

 α -AFP/NShS-S = Anti- α -fetoprotein Sepharose or normal sheep γ -globulin Sepharose.

†Pool of four samples from pregnancies with fetal disorders.

^{*}Percentage of the α-fetoprotein and zinc levels in eluate after the sample had been passed through anti-α-fetoprotein Sepharose/normal sheep γ-globulin Sepharose.

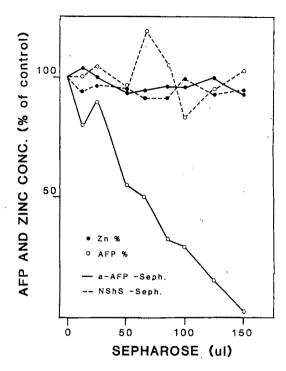


Fig. 3. Effect of the removal of α -fetoprotein on amniotic fluid zinc concentration. A fresh amniotic fluid sample (1 ml) was incubated with different amounts of anti– α -fetoprotein (α -AFP) Sepharose or normal sheep γ -globulin (NShS) Sepharose for 2 hours at room temperature.

els in pregnancies with various fetal malformations ranged from 1.8 up to 17.9 μ mol/L (median 5.0). The values were above 2.5 times the normal median (approximately the 97.5 percentile of controls) in 23 of the 29 cases (79%) with fetal malformations (Fig. 1, Table I).

Amniotic fluid zinc and α -fetoprotein levels showed a positive correlation (r = 0.78) (Fig. 2). Both levels were normal (below 2.5 times the median) in one case of polycystic kidneys. All other pregnancies with fetal malformations, including the six cases with the normal zinc value, had an increased α -fetoprotein concentration (Table I). The elevation of the zinc level was more marked than that of α -fetoprotein in three cases with malformations (Table II). Five samples from normal gestations had an elevated α -fetoprotein level (2.8 to 7.1 times the median) but a normal zinc concentration (Table II).

Incubation of fresh normal amniotic fluid with anti- α -fetoprotein immunoadsorbent removed α -fetoprotein but had no effect on the zinc concentration (Fig. 3). Also, passing of various types of samples through an anti- α -fetoprotein column had little effect on the zinc levels (Table III).

Comment

Zinc concentrations were above the 95 and 97.5 percentiles of controls in 86% and 79% of the early am-

niotic fluid samples from pregnancies with various fetal malformations, respectively. This confirms the original observation of Parkinson et al.,4 who found an increased zinc concentration in 12 gestations with anencephaly or open neural tube defect (range 2.0 to 7.2 μ mol/L) and in one case of gastroschisis (6.5 μ mol/L). The levels in our five cases of anencephaly ranged from 3.0 to 4.8 μ mol/L. Interestingly, these values were relatively low when compared to those observed in the majority of cases with fetal disorders. The highest values were found in two cases of hydrops fetalis (17.9 and 17.3 μ mol/L), and in congenital nephrosis, the values ranged from normal up to 15.2 μ mol/L.

Amniotic fluid zinc and α-fetoprotein levels showed a positive correlation. Our results, however, strongly suggest that this is not a result of the binding of zinc to amniotic fluid α-fetoprotein, as previously suggested.⁴ Removal of α-fetoprotein from various types of amniotic fluid samples had no clear effect on the zinc levels. It seems probable that the correlation between zinc and α -fetoprotein is caused by simultaneous leakage of α-fetoprotein and some zinc-binding protein from the fetus into amniotic fluid. Albumin and α2-macroglobulin are the major carriers of zinc in serum,11, 12 and both or either of these might be responsible for the elevation of amniotic fluid zinc levels in complicated pregnancies. The possibility that the fetus excretes some other zinc-binding protein, however, cannot be excluded at the moment.

 α -Fetoprotein was, in general, a more sensitive marker for fetal malformations than zinc. There were, however, a few cases with a more marked increase in the zinc concentration than in the α -fetoprotein level. This and the finding of normal zinc levels in pregnancies that were false positive on α -fetoprotein measurement suggest that the determination of zinc might be a useful adjunct to the α -fetoprotein assay, especially in cases with marginally elevated α -fetoprotein levels.

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Prevention of endomyometritis by local application of antibiotic solution during cesarean section

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Previous studies have demonstrated a reduction in the incidence of endomyor etritis by using irrigation with 1000 mi of saline as a diluent at cesarean section. In this study 2 gm of cefoxitin sodium in 20 ml of saline solution was used for local application to the uterus and abdominal wound in 100 patients undergoing emergency cesarean section; results were compared to those in 100 patients who received no antibiotic at operation. Six patients in the treatment group developed endomyometritis as opposed to 33 in the untreated group (p < 0.001) and hospital stay was reduced by almost 2 days (p < 0.001). Local use of cefoxitin sodium appears effective in reducing the incidence of endomyometrits. A prospective double-blind study, however, is imperative before definite conclusions can be made. (AM J OBSTET GYNECOL 1985;152:565-8.)

Key words: Endomyometritis, cesarean section, antibiotics

With the advancement of anesthesia and surgical techniques, cesarean section has become a relatively safe operation, with a threefold increase in the past decade. Cesarean section, while itself a relatively safe procedure, is associated with a high postoperative infection rate varying from 48% to 88% in the presence of labor with ruptured membranes. The major contributor to this morbidity is endomyometritis.

Recently there have been several reports showing the effective use of prophylactic antibiotic irrigation to reduce the infectious morbidity after cesarean section.⁵⁻⁷ In all the reports, however, 800 to 1000 ml of normal saline solution was used as a diluent for the antibiotic.

The objective of this investigation was to evaluate the effectiveness of a prophylactic antibiotic with only 20

ml of normal saline solution used as a diluent for local application to the operative site. The mechanism of action of intraoperative irrigation is complex, but it would appear that the high concentration of the drug applied directly to the site is more important than the actual .rrigation.⁴

Material and methods

One hundred patients with ruptured membranes undergoing emergency cesarean section were treated with local application of 2 gm of cefoxitin (Mefoxin, Merck Sharp & Dohme), as described below. The only patients excluded were those with a history of drug allergy or those who had received an antibiotic within 2 weeks prior to operation. No patient showed any untoward reaction to the drug.

Cefoxitin, 2 gm, was dissolved in 20 ml of normal saline solution and drawn up into two 10 ml syringes. The first 10 ml of the solution was sprayed into the fundal area of the uterine cavity after delivery of the placenta and after adequate contraction of the uterus had been obtained. After closure of the first layer of the uterus and during closure of the second layer of

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Table I. Demographic and preoperative data

	No. of	patients	-	
Variate	Control group	Cefoxitin group	p value	
Mean age (yr)	23.7	24.7	NS	
Race				
White	43	37		
Black	57	63	.NS	
Parity			•	
Primiparous	70	44		
Multiparous	30	56	0.01	
Monitoring				
External	57	37		
Internal	43	63	0.05	
No. of pelvic examinations				
1-4	70	58 ·		
5-8	29	39		
>8	1	3	, NS	
Duration of				
membrane				
rupture				
<6 hr	40	35	3.	
>6 hr	60	· 65	NS	

Table II. Postoperative infections

	No. of		
	Control groups	Cefoxitin group	p value
Total group	100	100	
Endomyometritis	28	6 .	0.00_{-}
Wound infection	3	2 .	NS
Both infections	5	.0	0.05

the uterus, a further 5 ml was sprayed along the uterine wound.

The last 5 ml of the solution was sprayed into the abdominal wound after closure of the fascia. At nc t me was any excess solution sucked out.

One hundred consecutive patients with ruptured membranes who underwent emergency cesarean section immediately prior to the treatment group were used as a comparison group.

All cesarean sections were performed by residents or faculty members and both groups were routinely followed up by the same surgeons and the same infection control nurse. Both groups were monitored daily for infectious morbidity. The diagnosis of endomyometritis was made, in the absence of physical or laboratory evidence of another source of infection, if the patient had a persistent temperature elevation above 38° C after the first postoperative day, foul-smelling lochia, and/or unusual uterine or parametrial tenderness.

Results

Table I provides the demographic data of the two groups studied. Both the treated (cefoxitin) and un-

Table III. Indications for cesarean section

	No. of		
Indication	Control group	Cefoxitin group	p value
Cephalopelvic disproportion	59	49	
disproportion Fetal distress	´ 28	31	NS
Breech	12	14	
Previous cesar- ean section	- 1	6 .	1

Table IV. Operative data

	No. of	patients	
Variate	Control group	Cefoxitin group	p value
Uterine incision			
Transverse	98	100	NS
Vertical	2	. 0	NS
Preoperative values			
Hemoglobin (gm/dl)	12.42	12.45	NS
Hematocrit (%)	38.10	38.30	NS
Postoperative values			
Hemoglobin (gm/dl)	10.40	10.90	0.01
Hematocrit (%)	31.90	33.10	0.03
Duration of operation		•	
<60 min	39	74	0.01
>60 min	61	26	0.01
Level of surgeon			
Postgraduate year 2-4	89	90	NS
>Postgraduate year 4	11	10	NS
Postoperative length of stay (mean days)	7.3	5.6	0.001

treated (control) groups were comparable in all respects except parity and monitoring technique. A larger number of the treated patients were internally monitored, yet they had a lower incidence of endomyometritis (Table II).

Indications for cesarean delivery are shown in Table III. Cephalopelvic disproportion includes all patients delivered by cesarean section because of failure of labor to progress. A slightly larger number of patients in the control group underwent cesarean section for cephalopelvic disproportion, but the difference was not statistically significant. The repeat cesarean sections were patients admitted in labor who were not candidates for a trial of labor or who refused.

Comparison of preoperative and postoperative hemoglobin levels showed that the change in both groups had a significant difference with a p value of 0.01 in favor of the cefoxitin group (Table IV). The shorter postoperative stay of 5.6 days for the treatment group compared to 7.3 days for the control group is highly significant, especially with problems such as cost of care and hospital beds. Total operative time was almost reversed in the two groups, with the control group taking

Table V. Incidence rates of endomyometritis (rate per 100 patients) for various risk factors*

		,	Patients with e	endomyometritis		
Variate		Control group		Cefoxitin group		
	Total No. of patients	%	Ns.	%	No.	p value
Indication	-					
Fetal distress	59	14.3	4	3.2	1	NS
Cephalopelvic dispropor-	108	33.9	20	10.2	5	0.01
tion						
Monitoring						
External	94	13.5	7	2.8	1	NS
Internal	106	39.5	17	7.9	5	0.001
Parity						
Primiparous	114	31.4	22	13.6	6	0.005
Multiparous	86	20.0	6	0.0	0	0.02
Duration of membrane	₩					
rupture						
<6 hr	75	12.5	5	2.8	1	NS
>6 hr	125	38.3	23	7.7	5	0.01
No. of vaginal examinations						
1-4	128	22.8	16	3.4	2 4	0.005
5-8	68	41.4	12	10.2	4	0.01
Skin incision						
Midline	99	24.4	10	5.2	3	0.05
Transverse	101	30.5	18	7.1	3	0.01
Transverse uterine incision	198	28.6	28	6.0	6	0.01
Duration of operation				•		
<60 min	113	28.2	11	6.7	5	0.01
>60 min	· 87	27.8	17	3.8	1	0.01

^{*}Statistical comparisons between the control and cefoxitin groups were conducted with the χ^2 test on the frequencies.

more time than the cefoxitin group. There is, however, no explanation for this.

When risk factors for endomyometritis were examined and compared (Table V), a decrease in morbidity was seen with most factors in the treatment group, except for those externally monitored, those with membranes ruptured <6 hours, or those undergoing abdominal delivery for fetal distress. These are, however, low-risk situations.

Increasing duration of ruptured membranes was associated with a corresponding increased risk of endomyometritis in the control group but not in the treated group (Fig. 1).

Comment

Several reports concerning the effect of antibiotic irrigation at cesarean section have shown a dramatic reduction in the rate of endometritis.4-7 In all these studies 800 to 1000 ml of saline solution was used as a diluent, and the uterus, bladder flap, and abdominal gutters were irrigated with the use of a bulb syringe. Force was used to create a jetlike effect while the irrigant was simultaneously suctioned. Rudd et al.6 have stressed the technique, while Levin et al.7 showed saline solution irrigation alone to be less effective than when an antibiotic was added. Duff et al.4 have suggested that at least part of the effect may be due to systemic absorption of the drug, with subsequent redistribution to

the surgical site. The value of the "irrigational" aspect of local antibiotic irrigation at cesarean section is uncertain.

A simpler technique than the one described previously was used to test the effectiveness of local application of a proved antibiotic. Cefoxitin, effective as a prophylactic drug in cesarean section, both parenterally8 and locally as an irrigating solution,7 was selected as the drug of choice. The concentration of cefoxitin in decidual tissue after uterine and peritoneal lavage with 2 gm/L has been shown to be almost three times greater (92.5 \pm 10.1 μ g/gm) than after intravenous administration of 2 gm $(36.9 \pm 10.5 \mu g/gm)$.

Burke,10 in his classical studies of intra-abdominal contamination, showed that there is a short definite period when antibiotics have their maximum suppressible effect. This period begins when bacteria gain access to the tissue and is over in 3 hours. While the time frame may be longer, local application of antibiotic to the wound site during operation may have some merit, as demonstrated by this and previously mentioned studies.

Using a historic control group, as in this study, is not ideal and would certainly not meet the most stringent scrutiny. Both groups, however, showed no statistical difference in age, race, number of pelvic examinations, or duration of ruptured membranes, were operated on by house staff at the same levels of training, and were

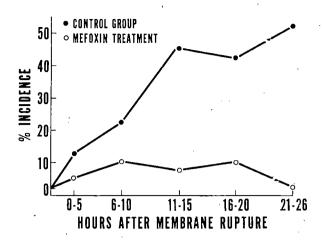


Fig. 1. Comparison of the incidence rates of endomyometric associated with the number of hours since membrane must be in the cefoxitin (Mefoxin) treatment group and the control group.

followed up by the same infection control nurse without any changes in the departmental protocol for the follow-up of patients who have undergone a cesareal section. This technique is simpler and far less time consuming than the lavage technique that has been used previously.

With external monitoring there was no statistical difference between the two groups. It would appear that local antibiotic "application" does provide some protection in the high-risk group, especially in those patients whose membranes are ruptured for more than 6 hours. There was also a distinct difference in those patients in whom the duration of labor was incresed because of cephalopelvic disproportion.

Despite a greater number of patients that were minitored internally in the treatment group, only 7.9% reveloped endomyometritis, as compared to 39.5% in the untreated group (p = 0.001).

Anemia after operation is generally accepted as being associated with a high rate of endomyometritis. Bath groups showed a postoperative decrease in hemoglobin values, with a p value of 0.01 in favor of the treated group. It is possible that this may have contributed an improved outcome; however, when the mean hemoglobin values of both groups were compared, the difference was still within laboratory error. Whether a fact this p value is significant is unclear.

Whether duration of operation per se contributes the incidence of morbidity after cesarean section is difficult to evaluate. Many variables may be associated with infection, such as duration of membrane rupture, anemia, and more than five pelvic examinations. 11 Duration

of operation was shorter in the treatment group and, therefore, possibly had some influence on the improved outcome of this group.

The method used in this study has had a very positive impact on the incidence of endomyometritis in our institution and has greatly decreased the work load of both residents and nurses in the postpartum wards. In the nontreated group, 33% of patients developed endomyometritis as compared to 6% in the treated group (p=0.001). This compares very favorably with the reports where lavage had been used. Hospital stay was reduced from 7.3 to 5.6 days (p=0.001), which also results in a savings in both time and money, 12 not to mention the direct value to the patient.

Despite the apparent success of local application of antibiotic during cesarean section, prospective studies comparing local application of antibiotic irrigation with antibiotic intravenous therapy and untreated control groups are imperative before any definite conclusions can be drawn.

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Bartholin's cyst: Marsupialization or aspiration?

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Of 34 patients with bartholinitis, two thirds had abscesses and one third had cysts. Aspiration provided not only relief but also material for bacteriologic culture. Ninety-five percent of the abscesses, half of them caused by gonorrhea, could be treated with penicillin and metronidazole. Cysts were not treated with chemotherapy. Cure rate was 85%. (AM J OBSTET GYNECOL 1985;152:569-70.)

Key words: Abscesses, bartholinitis, cysts, gonorrhea, marsupialization

A swelling of the Bartholin's gland presents the clinician with two problems: Is it a cyst or is it an abscess? Should it be treated with antibiotics or operation or both? The answer to the first question is usually a guess, as pain and size are unreliable guides. The answer to the second is frequently empirical—a trial of antibiotics followed by operation if there is no improvement.

Forty years ago the recommended treatment was complete excision of the gland. Then, in 1948, in New York, Davies' described a simple technique of marsupialization and packing of the cavity with iodoform gauze. By 1966 Mathews' was able to declare this "an established method of treatment," albeit with a 13% failure rate.

The question then is not whether the operation is successful but when it should be performed. For example, could it be avoided altogether?

A rational approach would be to aspirate the contents of the swelling and submit them to bacteriologic analysis for identification and antibiotic sensitivity. A decision on chemotherapy could then be made and the outcome awaited. Failure to respond would indicate the need for operation.

This would contrast with the more customary approach in which culture material is finally acquired only at the time of operation. At this late stage, analysis is of doubtful value: It has frequently been compromised by a preceding course of antibiotics. The patient has usually left the hospital by the time the results are available. The operation is, in itself, definitive treatment.

Patients and methods

From 1980 to 1983, 34 patients aged 16 to 45 years with bartholinitis were investigated in the following manner: A forefinger was introduced into the vagina and the swelling gently compressed between finger and

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Table I. Bacteriologic results

No. of patients (n = 34)	Organism	Sensitivity to penicillin
11	Neisseria gonorrhoeae	Yes
2	β-Hemolytic strep- tococci	Yes
1	Streptococcus pneu- moniae	Yes
1	Streptococcus viridans	Yes
1	Nonhemolytic streptococci*	Yes
1	Anaerobic strepto- cocci*	No (metro- nidazole)
I	Staphylococcus aureus	No (erythro- mycin)
3	Sterile pus	
13	Mucus	***************************************

^{*}Also had Bacteroides fragilis (sensitive to metronidazole).

thumb. At the maximum point of fluctuation a size 19 needle was inserted and the contents aspirated for analysis. Performed in the office, without anesthesia, the procedure was well tolerated and afforded immediate, symptomatic relief.

Results

The appearance of the aspirate provided a ready method of distinguishing between cysts and abscesses, thereby identifying those patients who required antibiotics. Classification was simple and based on the presence or absence of pus cells. Cysts contained thick, translucent mucus, which was more difficult to aspirate than were the abscesses whose contents were always less viscous and frankly infected.

Table I shows the bacteriologic findings. Twenty-one of the 34 patients were adjudged to have abscesses, 11 of them caused by gonorrhea. All 21 patients were treated with metronidazole, 400 mg twice a day, and penicillin (or erythromycin), 250 mg four times a day, for 1 week and then reviewed. The patients with gonorrhea were also given a stat dose of 1 gm of probenecid and 3.5 gm of ampicillin, early diagnosis having been made by microscopy.

Follow-up

The overall success rate, marked by the absence of swelling and discomfort and the appearance of a freely draining duct, was the same in both groups-85% (three abscesses and two cysts failed to resolve and were marsupialized). Average follow-up period was 10 months, with four patients who were lost to follow-up.

Comment

Whatever the mechanism of duct blockage in both cysts and abscesses, resolution can be predicted confidently in a high proportion of patients if the conservative approach described above is followed. Operation need not be first-line treatment. However, the range of organisms isolated demonstrates the importance of bacterial identification if therapy is to be planned and modified appropriately.

The high incidence of gonorrhea among the patients with abscesses was unexpected—for most patients a painful swelling was the only complaint. Among this group, aspiration had the added advantage of identi-

fying promptly those women whose male partners would require investigation.

Of concern for the future is that, although all these gonococcal abscesses responded to ampicillin, the increasingly common strain of penicillinase-producing Neisseria gonorrhoeae is now causing problems that require different antibiotics such as spectinomycin or one of the cephalosporins. Therefore early identification is even more important.

In a revealing comment on gynecologic teaching in the 1930s, Davies1 tells us that he "was taught to assume that each case, more likely than not, was the result of a venereal infection." Are sexual habits today so markedly different?

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Maternal virilization in pregnancy due to an unclassified sex-cord stromal neoplasm

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Virilization in pregnancy has been reported with various ovarian neoplasms. Presented is a case of maternal virilization resulting from an unclassified sex-cord stromal neoplasm. (AM, J OBSTET GYNECOL 1985;152:570-2.)

Key words: Virilizing neoplasms, pregnancy, sex-cord stromal neoplasms, ovarian neoplasms, androgens

We report an unusual case of maternal virilization in pregnancy due to an unclassified sex-cord stromal neoplasm.

Case report

A 21-year-old woman, para 0-0-0-0, presented at 24. weeks' gestation with hirsutism, acne, and deepening of the voice for the previous 8 weeks. She denied the ingestion of any drugs during this pregnancy.

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Examination revealed marked hirsutism and acne, with loss of temporal hair. No clitoromegaly was noted. The corpus was 24 weeks in size, and fetal heart tones were present.

Sonography disclosed a 26-week intrauterine pregnancy, with a solid 7 by 7 cm mass in the left adnexa; the right ovary was normal.

Endocrine data included: total serum testosterone, 90 ng/dl ("free" testosterone, 0.4 pg/ml); androstenedione, 620 ng/dl; 17α-hydroxyprogesterone, 500 ng/ dl; dehydroepiandrosterone sulfate, 50 µg/dl; urinary 17-ketosteroids, 8.4 mg/24 hr; and urinary 17-hydroxycorticoids, 5.4 mg/24 hr.

At operation, a 7 by 8 cm left ovarian mass was found to be adherent to the cul-de-sac and posterior surface of the corpus. Oophorectomy was performed on the left side. The postoperative course was benign.

Repeat serum androgen assays on the third post-

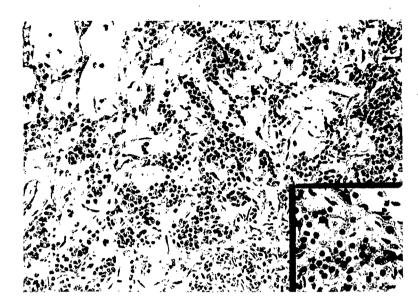


Fig. 1. Clusters of cells separated by a loose edematous stroma. (Hematoxylin and eosin. ×40.) Inset: Tumor cells form syncytial clusters. Nuclei are round and oval, uniform in size. Cytoplasm is pale eosinophilic, granular, and foamy. (Hematoxylin and eosin. × 100.)

operative day revealed a total serum testosterone of 31 ng/dl; androstenedione, 77 ng/dl; 17α-hydroxyprogesterone, 177 ng/dl; and dehydroepiandrosterone sulfate, 30 µg/dl. Over the next few weeks, the patient reported disappearance of acne and cessation of growth of hair. Six weeks postoperatively, total serum testosterone was 10 ng/dl; androstenedione, 70 ng/dl; 17α-hydroxyprogesterone, 110 ng/dl; and dehydroepiandrosterone sulfate, 52 µg/dl. At 36 weeks, spontaneous labor ensued, and a 2,505 gm nonvirilized female infant was delivered vaginally.

Gross description. The tumor was encapsulated, and measured 6.5 by 4 by 2 cm. At one edge, normal ovarian structures were seen, and the tumor appeared to arise from the hilus. The cut surface was partly solid, partly cystic, and necrotic.

Microscopic description. Clusters of cells haphazardly distributed in a loose stroma were seen. They varied in shape from oval to stellate and squamoid (Fig. 1). The nuclei were rounded and oval, and the cytoplasm was granular and foamy (Fig. 1, inset). There were no formed tubules or pattern suggestive of tubules. No Leydig cells were seen. Reticulum stains revealed a reticular stroma that encompassed from two to six cells in interlacing bundles. Electron microscopy disclosed cells with grooved nuclei. Lipid of differing densities was abundant in the cytoplasm. Mitochondria were abundant, and in some cells the mitochondria contained electron-dense deposits, suggestive of lipid synthesis. There was abundant glycogen in the cytoplasm.

Diagnosis. The diagnosis was sex-cord stromal tumor, unclassified.

Comment

In a survey of 44 virilizing ovarian tumors in pregnancy reported through 1973, Verhoeven et al.1 re-

viewed eight luteomas, eight arrhenoblastomas, six Krukenberg tumors, five Leydig cell tumors, five granulosa-theca cell tumors, three mucinous cystadenomas, one mucinous cystadenocarcinoma, and one included case of hyperthecosis.

The disappearance of maternal symptoms and signs and the prompt reduction in serum testosterone, androstenedione, and 17α-hydroxyprogesterone subsequent to removal of the tumor confirm its hormonal activity. The lack of virilization of the fetus may have been due to a number of factors. A female fetus is virilized only 50% of the time when maternal virilization is associated with luteoma of pregnancy. Placental aromatization of androgens to estrogens may be protective both in normal pregnancies and in pregnancies complicated by hyperandrogenic states. The nature of the androgens elaborated by the tumor may be important. The predominant androgen measured in this patient was a relatively weak one, androstenedione, and the elevation present was only moderate (620 ng/dl). Biologically active or "free" testosterone was normal, and total testosterone was slightly elevated (90 ng/dl), compatible with pregnancy and reflecting the estrogendependent increase in sex-hormone binding globulin. Finally, the onset of hyperandrogenemia in relationship to the embryogenesis of the external genitalia may be critical, with exposure to androgen prior to 12 weeks' gestation being more likely to virilize the female fetus than exposure after that time. Maternal virilization in this patient was reported as occurring historically between 16 and 24 weeks' gestation. The source of androgen was surgically extirpated, so that no further exposure to androgen occurred between 26 weeks and delivery 10 weeks later.

The histologic features of this virilizing neoplasm are of interest in that no tubules or patterns suggestive of tubules were seen. Leydig cells were absent. The reticular stroma containing interlacing bundles of tumor cells is suggestive of that seen in granulosa cell tumors. Lipid synthesis is suggested by the abundant crtoplasmic lipid and the presence of electron-dense ceposits in mitochondria.

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Fluorescence polarization values of amniotic fluid collected from the vagina after rupture of the membranes

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Transvaginal collection of amniotic fluid and the use of a reliable test for fetal lung maturity will provide information needed in the management of patients with premature rupture of the membranes. (AM J OBSTET GYNECOL 1985;152:572-3.)

Key words: Fluorescence polarization, vaginal pool amniotic fluid, fetal lung maturity, lecithin/sphingomyelin ratio

The study of amniotic fluid to assess fetal lung maturity may be useful in the clinical management of patients with premature rupture of the membranes. Performing amniocentesis after rupture of the membranes may be difficult, whereas amniotic fluid from the vaginal pool may be readily obtained in these patients. The validity of using vaginal pool samples of amniotic fluid to analyze fetal lung maturity indices has been cuestioned for a number of reasons. Amniotic fluid collected from the vaginal pool may be diluted by vaginal secretions or contaminated by blood and epithelial cells. The lecithin/sphingomyelin ratio of amniotic fluid from the vaginal pool has been reported not to correlate with the lecithin/sphingomyelin ratio in the fluid from the amniotic sac. However, the presence of phosphatidylglycerol in vaginal pool samples reliably reflected the presence of phosphatidylglycerol in the amniotic sac.1

Fluorescence polarization analysis of the amniptic fluid has proved to be a rapid and reliable means of predicting fetal lung maturity.2 Fluorescence polar za-

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tion measures the lipid microviscocity of cell membranes and liposomes. The procedure requires mixing 0.5 ml of amniotic fluid and a hydrocarbon probe DPH (1,6 ciphenyl 1,3,5 hexatriene). The results are determined by a fluorescent polarimeter (Microviscosimeter, Model VM-1, Elssint, Inc., Hackensack, NJ). The entire process can be performed in 45 minutes. A fluorescence polarization result of less than 0.336 correlates with a lecithin/sphingomyelin ratio of greater than 2 and the lack of respiratory distress syndrome. The current study was undertaken to compare the fluorescence polarization values of amniotic fluid collected from the vaginal pool with those of fluid obtained from the amniotic sac.

Material and methods

Amniotic fluid was collected in the following manner from 19 patients in labor at term. Prior to amniotomy, transmembranous needle aspiration of 10 ml of amniotic fluid through the dilated cervix was performed with a No. 20 gauge spinal needle. Thirty minutes later, 5 to 10 ml of fluid was collected from the vaginal pool. Fluore: cence polarization analyses were performed on the paired samples.

Results

The mean fluorescence polarization value of fluid obtained from the amniotic sac was 0.261 ± 0.0577 (SD), and the mean fluorescence polarization value of

the fluid from the vaginal pool was 0.271 ± 0.0511 (SD). Linear regression analysis determined a correlation coefficient of 0.906, which is significant at the p < 0.001 level (Fig. 1). Seventy-nine percent of the 19 samples had a higher fluorescence polarization value on the vaginal pool fluid than that on the matched fluid from the amniotic sac. All vaginal pool samples that were not contaminated with blood had higher fluorescence polarization values than those of corresponding amniotic fluid samples. The higher fluorescence polarization value of the vaginal pool fluid may possibly be interpreted as a false positive prediction of fetal lung immaturity. However, the fluorescence polarization values in all sets of clear fluid were less than 0.336, thus indicating fetal lung maturity.

Seven of the vaginal pool samples of fluid contained blood with a hematocrit of 1% or less. Forty-three percent of these samples had fluorescence polarization values lower than those of matched amniotic sac fluid. Blood-tinged vaginal pool samples do not show any consistent relationship to the amniotic sac fluid samples and should not be used to assess fetal pulmonary maturity.

Comment

The fluorescence polarization values determined on samples of clear amniotic fluid collected from the vaginal vault after rupture of the membranes reliably correlates with the values determined on fluid obtained from the amniotic sac. A mature fluorescence polarization value obtained from vaginal pool clear amniotic fluid should be associated with fetal lung maturity. The correlation of vaginal pool fluid in the preterm pregnancy with ruptured membranes has not been addressed by this study. Both the effect of longer duration of fluid contact with the vagina and the presence of

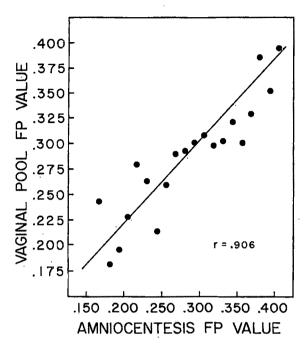


Fig. 1. Correlation of vaginal pool and amniotic sac fluorescence polarization (FP) values. Correlation coefficient = 0.906, p < 0.001.

immature lung indices may alter the reliability of this method. Further investigation of this relationship will be undertaken in the preterm gestation.

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The natural history of ventriculomegaly in a fetus without obstructive hydrocephalus

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Fetal ventriculomegaly is an abnormal increase in the size of the lateral cerebral ventricles. Presented here is a case of fetal ventriculomegaly in which there was spontaneous intrauterine improvement in its severity. (Am J OBSTET GYNECOL 1985;152:574-5.)

Key words: Hydrocephalus, ventriculomegaly, ultrasonics, antenatal diagnosis, fetus

Fetal ventriculomegaly is an abnormal increase in the size of the lateral cerebral ventricles. Recently, the lateral ventricle/hemispheric width ratio has been utilized as the standard by which antenatal ventricular size is assessed,1 and the diagnostic accuracy of this parameter in the prediction of fetal ventriculomegaly has been established.² Cross-sectional studies, however, have not provided information concerning the natural history of fetal ventriculomegaly. Although longitudinal studies of neonatal ventriculomegaly have been reported, their relevance to the fetal condition is uncertain. The purpose of this paper is to describe a case in which spontaneous intrauterine improvement in the severity of fetal ventriculomegaly occurred.

Case report

The patient was 21 years old, gravida 2, para 1-0-0-0, with unremarkable medical, family, and obstetric histories. Diagnostic ultrasound studies were performed at 20 weeks of gestation to assess gestational age. The lateral ventricle/hemispheric width ratio of 88% was abnormally increased. No other structural anomalies were identified; the amniocyte karyotype was 46,XY.

During eight subsequent sonographic examinations, the lateral ventricle/hemispheric width ratios continued to decrease (Fig. I) and the weekly ratios from 341/2 to 361/2 weeks were within the normal range. Throughout gestation, biparietal diameter, femur length, and abdominal circumference measurements showed appropriate growth. Induction was performed at 361/2 weeks of gestation because of the development of bilateral

Fig. 1. Lateral ventricle/hemispheric width (LV/HW) from 20 to 361/2 weeks of gestation superimposed on nomogram of lateral rentricle/hemispheric width by gestational age as modified from Jeanty et al.1

hydronephrosis. There was a spontaneous vaginal delivery of a 2700 gm male infant with Apgar scores of 3 at 1 minute and 6 at 5 minutes.

Head circumference at the time of birth was 33.5 cm, appropriate for gestational age. Fontanelles were soft and cranial sutures were overlapped. Computed axial tomography revealed moderate dilatation of the lateral ventricles and third ventricle and absence of the corpus callosum. The fourth ventricle was normal in size, and there was marked prominence of the subarachnoid space over the frontal lobes and sylvian fissures.

After birth it was recognized that the perinatal asphyxia was due to a tracheoesophageal fistula and tracheal malacia. At the time of this report the infant was 71/2 months of age with moderate delay in gross motor

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Comment

Improvement in the severity of ventriculomegaly was documented by longitudinal sonographic studies in this case. Although ventricular size was increased in relation to brain substance early in gestation, this disproportion regressed as the brain grew during the second half of gestation. This finding parallels the decrease in lateral ventricle/hemispheric width ratio throughout gestation, which occurs in normal pregnancies.1 The presence of continuing ventriculomegaly in postnatal life indicates, however, that fetal brain growth had not been normal.

The etiology of the ventriculomegaly is uncertain. Although the moderate dilatation of the lateral ventricles and third ventricle in conjunction with a normal fourth ventricle suggests an intermittent or incomplete aqueductal stenosis, the prominence of the subarachnoid space over the frontal lobes and sylvian fissures is more indicative of brain dysgenesis or brain atrophy. In addition, there were no clinical signs of increased intracranial pressure throughout 71/2 months of postnatal life to support a diagnosis of obstructive hydrocephalus. It is uncertain whether the delays in gross motor function were due to brain dysgenesis or to perinatal asphyxia. It is possible that the pathologic condition of the brain may have resulted from a neuronal migrational disorder associated with absence of the corpus callosum.

Consideration of the possibility of brain dysgenesis without obstructive hydrocephalus is essential when placement of a ventriculoamniotic shunt is contemplated. Although the therapeutic benefit of ventriculoamniotic shunt placement is uncertain, it may be of value for selected cases of obstructive fetal hydrocephalus. It is most unlikely, however, that this intervention would be helpful if diminished brain substance were the cause and not the result of the ventriculomegaly. Progressive ventriculomegaly should therefore be documented if shunt placement is considered.

In conclusion, clinical data concerning the etiology, frequency, and natural history of ventriculomegaly are greatly needed. At the present time it is uncertain how often ventriculomegaly spontaneously resolves, how to distinguish slightly enlarged ventricles in a normal fetus from pathologic ventriculomegaly, and how to differentiate the ventriculomegaly caused by brain dysgenesis from that caused by obstructive hydrocephalus. With improvements in diagnostic imaging, quantification of the subarachnoid spaces around the fetal brain or definition of other sonographic markers of brain dysgenesis might permit more precise identification of this condition.

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Severe hypoglycemia associated with HELLP syndrome

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Presented is a case of the syndrome of hemolysis, elevated liver enzymes, and low platelet counts, in which severe maternal hypoglycemia occurred 8 hours after delivery. It is proposed that the hepatic pathologic features in this case were severe enough to interfere with glycogenolysis and glyconeogenesis. (AM J OBSTET GYNECOL 1985;152:576-7.)

Key words: HELLP syndrome, preeclampsia, hypoglycemia

This article describes a case of severe maternal hypoglycemia associated with the syndrome of hemolysis, elevated liver enzymes, and low platelet counts (HELLP).

Case report

R. C., an 18-year-old primigravid woman, presented at 30 weeks' gestation with uterine contractions, vaginal bleeding, and a 2-week history of nausea, vomiting, and epigastric pain. Her prenatal course was remarkable only in that a twin gestation had been diagnosed 7 weeks previously. Blood pressure values had been normal at each prenatal visit, and proteinuria had been absent. On admission, she was found to have scleral icterus, jaundice of mucous membranes, mild epigastric tenderness, pretibial edema, hyperreflexia, a blood pressure of 156/108 mm Hg and proteinuria (I+). Fetal heart tones could not be heard, and real-time sonography confirmed the death of both fetuses. Admission laboratory tests included: hematocrit, 43%; placelet count, 156,000/mm³; fibrinogen, 37 mg/dl; amylase, 69 U/L; and uric acid, 13.7 mg/dl. Liver function tests included: serum glutamic oxaloacetic transaminase, 140 U/ml; serum glutamic pyruvic transaminase, 97 U/ ml; lactate dehydrogenase, 776 U/dl; alkaline phosphatase, 303 U/ml; total bilirubin, 11.3 mg/dl; and serum albumin, 3.1 gm/100 ml.

An intravenous infusion of lactated Ringer's solution was started, and intravenous magnesium sulfate therapy was instituted. Three hours after admission, the patient was delivered of a 2,160 gm stillborn male infant and an 1,800 gm stillborn female infant. Blood lost at delivery was estimated at 600 ml.

During the remainder of the patient's hospitalization, the diastolic blood pressure values remained between 90 and 100 torr. By 3 hours after delivery, the hema-

tocrit had fallen to 31.6%, and schistocytes were found on peripheral smear.

Eight hours after delivery, the patient suddenly became unresponsive and developed decerebrate posturing. The blood pressure was 140/90 mm Hg. Arterial blood gases included: pH, 7.43; PCO₂, 40; and PO₂, 87. The serum magnesium level was 5.3 mEq/L; calcium, 9.3 mg/dl; fibrinogen, 23 mg/dl; platelet count, 109,000/mm³; and plasma glucose, 3 mg/dl. She became immediately responsive after the intravenous administration of 100 gm of glucose. All intravenous lines and bags were analyzed for insulin, and no evidence of exogenous insulin was found. Thereafter, all intravenous infusions contained 5% dextrose. There were no subsequent episodes of hypoglycemia.

By 18 hours after delivery, the platelet count had fallen to 94,000/mm³, and the fibrinogen level to 14 mg/dl. Levels of clotting factors V and VII were 20% and 11%, respectively, thus implying a failure of hepatic synthesis of these factors as a major contributor to the coagulopathy.

Although the patient continued to do well clinically, with no excessive vaginal bleeding, by the fourth postpartum day, she continued to have depressed levels of hepatically produced clotting factors, including factors V (53%), VII (12%), IX (25%), and X (37%). The total bilirubin was 9.3 mg/dl, with a direct component of 5.9 mg/dl. The serum albumin was 1.7 gm/100 ml. The platelet count had increased to 166,000/mm³. Hepatitis B screening tests (HBSAg, HBSAb, HBCAb, HACAb) were negative. A sonogram of the gallbladder was normal. The intravenous infusions were discontinued, and subsequent plasma glucose values were in the normal range. By the sixth postpartum day, all coagulation tests produced normal values.

Six weeks after delivery, all findings on liver function tests, coagulation studies, and a fasting plasma glucose test were normal.

Comment

At the time of admission, this patient was thought to have the HELLP syndrome and twin fetal death. HELLP syndrome, as described by Weinstein, is characterized by thrombocytopenia, hemolysis (microangio-

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pathic anemia), and abnormal liver enzymes in the preeclamptic or eclamptic patient. Killam et al.2 had earlier described five similar cases of pregnancy-induced hypertension complicated by acute liver disease and disseminated intravascular coagulation. One of Killam et al.'s patients was described as having moderate scleral icterus. Both Weinstein and Killam et al. recommended aggressive management with expedient delivery. Thus, our patient was treated with magnesium sulfate, and delivery was assured. Post partum, she was found to be in a hypoglycemic coma approximately 7 hours after intravenous fluid had been changed from glucose-containing solution to lactated Ringer's solution. Known causes of hypoglycemia, including exogenous insulin administration, an insulin-secreting tumor, and panhypopituitarism, were excluded. Hypoglycemia has not been described as being part of the HELLP syndrome.

Hypoglycemia, however, has been well described as part of the syndrome of acute fatty liver of pregnancy. The exact relationship between preeclampsia and acute fatty liver of pregnancy remains undetermined. Possibly, a spectrum of hepatic damage ranging from preeclampsia to HELLP syndrome to acute fatty liver of pregnancy may occur in pregnancy. Indeed, Pockros et al.3 report that, in cases of acute fatty liver of pregnancy, "early recognition of hypoglycemia and prevention of coma by dextrose infusions has significantly lowered the number of patients who became encephalopathic." In any case, it appears that early delivery and meticulous supportive care are the mainstays of therapy.

The hepatic pathologic features in this case of HELLP syndrome appear to have been severe enough to have interfered not only with the production of hepatically produced coagulation factors, but also with glycogenolysis and glyconeogenesis. Careful attention to hypoglycemia and clotting factors is essential. This case illustrates in particular the need for continuous infusions of dextrose and monitoring of serum glucose.

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Immune complexes in preeclampsia and normal pregnancy

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We determined in normal nonpregnant (group I) and normal pregnant (group II) women and in patients with preeclampsia (group III): (1) immunoglobulins and complement C3b associated with polymorphonuclear leukocytes and platelet surfaces in an attempt to evaluate the interaction in vivo of immune complexes with the membranes of these cells; (2) the occurrence of circulating immune complexes; (3) the serum levels of immunoglobulins, C3, and C4; and (4) the plasma levels of complement C3d. In patients with preeclampsia (group III), the percentages of polymorphonuclear leukocytes and platelets positive for membrane-bound IgG, IgM, IgA, and C3 were significantly higher than the percentages in groups I and II. In group III, there also was a significant increase in circulating immune complexes, as compared to groups I and II. However, circulating immune complexes were also present in significant amounts in normal pregnancy (group II). The plasma levels of complement C3d were markedly increased in the most severe cases of preeclampsia. LAM J OBSTET GYNECOL 1985;152:578-83.)

Key words: Immune complexes, normal pregnancy, preeclampsia

The syndrome of preeclampsia is more common during first pregnancies, tends to occur in certain families, and can develop subsequent to changes in mating partners; consequently, the suggestion has been made that preeclampsia may be immunologically mediated. The deposition of immunoglobulins and complement in the placentas of preeclamptic patients occurs in a manner similar to the inappropriate immune response which occurs in rejected renal allografts.1 Furthermore, Petrucco et al.2 demonstrated deposits of IgM, IgG, and complement in the glomeruli of women with preeclampsia. The claim has been made that circulating immune complexes are of some relevance in the tissue injury that occurs in preeclampsia, but their association with the syndrome is controversial.3-5 Previous publications have shown that circulating immune complexes interact in vivo with polymorphonuclear leukocytes through surface receptors on the polymorphonuclear leukocytes for the Fc-fragment of the complexed immunoglobulins or the C3b complement fraction⁶; circulating immune complexes also interact with platelets through the Fc-fragment.7

The present study in patients with preeclampsia was undertaken to determine: (1) immunoglobulins and complement C3b associated with polymorphonuclear leukocytes and platelet surfaces in an attempt to evaluate the interaction in vivo of immune complexes with the membranes of these cells; (2) the occurrence of circulating immune complexes; (3) signs of intravascular complement activation as detected by the levels of C3d; and (4) the serum levels of immunoglobulins, C3, and C4.

Subjects and methods

Subjects. Three groups of subjects were studied: 30 normal nonpregnant women (group I), 30 normal pregnant women (group II), and 38 pregnant women with preeclampsia (group III).

The diagnosis of "preeclampsia" was made according to the following criteria: (1) blood pressure higher than 140/90 mm Hg after 20 weeks of pregnancy in previously normotensive patients; (2) urinary protein concentration over 300 mg/L; and (3) evidence of abnormal fluid retention. Only two patients developed eclamptic seizures. The clinical data on groups II and III are reported in Table I, that shows the range and mean values ± SD of ages of all pregnant women, the increase in their body weight during pregnancy, the mother's systolic and diastolic blood pressure values in the third trimester, the weeks of pregnancy at parturition, and the fetal weights and Apgar score; the number of primigravid women, the number of deliveries

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Table I. Clinical data on pregnant women and their offspring

. Clinical data	Normal pregnant women (group II) $n = 30$	Pregnant women with preeclampsid (group III) n = 38
Age (yr)	·27 ± 4.7	28 ± 5.0
0 4 7	(20-38)	(18-41)
Primigravid (No.)	11	20
Increase in body weight (kg)	13 ± 4	15 ± 5
, 8 . 0,	(7-20)	(7-26)
Systolic blood pressure (mm Hg)	122 ± 8	161 ± 17
,	(110-140)	(130-200)
Diastolic blood pressure (mm Hg)	76 ± 5	103 ± 10
1	(70-90)	(80-125)
Weeks of pregnancy at parturition	40 ± 1.2	38 ± 3.3
. 0 , .	(37-42)	(30-42)
Deliveries by cesarean section (No.)	15	23
Fetal weight (gm)	3245 ± 396	2944 ± 801
3 .5 .	(2190-3900)	(770-3920)
Apgar score	8.4 ± 1.1	6.6 ± 2.7
	(5-9)	(0-9)
Male-to-female sex ratio	1.3	1.2

by cesarean section, and the male-to-female sex ratio. Stillbirths occurred in two cases of preeclampsia. The normal nonpregnant women of group I matched the normal pregnant women of group III for age and parity. All subjects were negative for antinuclear and anti-DNA antibodies, cryoglobulins, and rheumatoid factor. In groups II and III, blood was drawn before the onset of labor.

Polymorphonuclear leukocyte immunohistologic technique. Polymorphonuclear leukocytes were separated and purified as previously described. 6-8 Briefly, blood was collected in 20 ml plastic tubes that contained 5 mmol/L ethylenediaminetetraacetic acid (EDTA), pH 7.2, and centrifuged at 700 g for 20 minutes. Plasma and the buffy coat were removed, and the polymorphonuclear leukocyte-rich layer was aspirated and resuspended in a 1:2 proportion with 2.5% gelatin (Difco) in Tris-buffered physiologic saline solution, pH 7.2. After erythrocyte sedimentation, polymorphonuclear leukocytes were washed three times in Tris-Tyrode's bovine serum albumin (TTBSA) buffer devoid of Ca++ and Mg++ (TTBSA-no Ca++-no Mg++). The composition of TTBSA was as follows: potassium chloride, 2.6 mmol/L; MgCl₂6H₂O, 1.0 mmol/L; sodium chloride, 1.37 mmol/L; CaCl₂6H₂O, 1.3 mmol/L; glucose, 0.1%; Tris, 1.0 mmol/L; 0.25% bovine serum albumin (Behring Institut). All solutions were buffered at pH 7.4, at 22° C, unless otherwise stated.

Cell viability was assessed by Y eosin exclusion.

Polymorphonuclear leukocytes (5 × 106/ml) were resuspended in TTBSA-no Ca++-no Mg++ that contained EDTA, 1.0 mmol/L, to prevent phagocytosis.

The presence of immunoglobulins and/or C3 on polymorphonuclear leukocyte surface was ascertained with the use of monovalent Fab fragment obtained from rabbit anti-human IgG, IgM, IgA, and C3 fraction of the complement, conjugated with fluorescein isothiocyanate.8 The excess of antisera was eliminated by three washings with TTBSA-no Ca++-no Mg++, with intermediate centrifugations at 300 g for 10 minutes.

To estimate the amount of free receptors, a phagocytosis assay was performed with the use of complement C3b-activated baker's yeast particles (BYP) (C3b-BYP) as substrate, in order to explore C3b surface receptors. The nuclei of polymorphonuclear leukocytes were stained with 0.075% toluidine blue (Merck) in 30% ethanol brought to pH 3.4 ± 0.2 with glacial acetic acid. With the use of this staining mixture, which is not autofluorescent and fully hemolytic, the cells could be easily recognized on the basis of nuclear morphologic features, and complete differential counts of the stained cells could be performed. During the search for membrane-bound immunoglobulins and/or C3, each microscopic field was examined alternatively under normal and ultraviolet light, and the percentage of polymorphonuclear leukocytes that showed membrane fluorescent spots was scored. In studies of polymorphonuclear leukocyte free receptors for C3b, polymorphonuclear leukocytes exposed to toluidine blue were scored with the use of a light microscope and were considered to be phagocytic when one or more C3b-BYP were internalized.

On the basis of previous findings obtained with a control population (n = 980), unmatched for age and sex, the test was considered to be positive when more than 5% of the polymorphonuclear leukocytes showed membrane deposits of immunoglobulins and less than 80% were phagocytic.

Platelet-associated immunoglobulins and C3. Platelet-rich plasma obtained from whole blood as described above for polymorphonuclear leukocytes was aspirated and recentrifuged at 1500 g for 15 minutes. Platelets were resuspended in TTBSA-no Ca++-no Mg++, washed five times in the same buffer, with intermediate

Table II. Detection of immune-complexes in groups I, II, and III, by different techniques (values expressed as mean \pm SEM)

	% Polymorphonuclear le tkocytes with immunoglobins and C3 deposits				% Polymorphonuclear
	- IgG	IgA .	IgM	. С3	leukocytes phagocytozing C3b-BYP
Group I (n = 30)	0.7 ± 0.3	0.5 ± 0.2	0.4 ± 0.3	0.0	90.0 ± 1.3
Group II	0.6 ± 0.2	0.3 ± 0.1	0.4 ± 0.2	0.1 ± 0.1	86.3 ± 1.2
(n = 30)	NS*	NS*	NS*	NS*	p < 0.05*
Group III	6.0 ± 1.1	1.8 ± 0.5	2.3 ± 0.6	0.5 ± 0.2	78.1 ± 2.2
(n = 38)	p < 0.001†	p < 0.05†	p < 0.01†	NS†	$p < 0.01\dagger$
,	p < 0.001‡	NS‡	p < 0.02‡	p < 0.02‡	p < 0.001‡

Group I = normal nonpregnant women. Group II = normal pregnant women. Group III = pregnant women with pre-eclampsia.

C3b-BYP = C3b-opsonized baker's yeast particle: C1q INH = C1q latex agglutination inhibition test. KIE = Conglutinin immunoenzymatic test.

NS = Not significant ($p \ge 0.05$).

centrifugation (1500 g for 15 minutes), and finally resuspended in TTBSA-no Ca⁺⁺-no Mg⁺⁺ supplemented with EDTA 1.0 mmol/L. The presence of immunoglobulins and C3 bound to platelet membranes was investigated with a procedure similar to that described above for polymorphonuclear leukocytes. In each microscopic field, platelets and their membrane-bound immunoglobulins and/or C3 were recognized, respectively, by phase-contrast and ultraviolet-light microscopy. As for polymorphonuclear leukocytes, the test was considered to be positive when more than 5% of the platelets showed membrane deposits of mmunoglobulins.

C1q latex agglutination inhibition (C1q INH) test. The sera of the patients were tested for their inhibitory activity on the agglutination of IgG-coated polystyrene particles (latex) by C1q according to Lurhuma et al.⁹ as modified by Caligaris Cappio et al.¹⁰ Sera from patients and healthy subjects were tested blind in duplicate. Agglutination was measured by monitoring the modifications of light transmittance of latex particles stirred at 900 rpm at 37° C in a glycine—sodium chloride buffer, pH 8.2, in an Elvi 840 aggregometer, gain 1, and recorded by a OmniScribe recorder, voltage 0.1. Fesults were expressed as percentage inhibition of agglutination according to Levinsky and Soothill.¹¹ Levels of inhibition above 20% were considered to be positive.

Conglutinin immunoenzymatic (KIE) test. The KIE test was performed as described by Manca et al. with the use of an enzymatically active antigen (β -galactosidase purified from *Escherichia coli*) and antibody (donkey antiserum to β -galactosidase) complex as probes to measure circulating immune complexes in the sera from groups I, II, and III by competition for the binding with bovine conglutinin linked to polystyren= particles. To detect the probe complex, the θ -nitrophenyl

galactopyranoside was used as enzymatic substrate, since it is hydrolyzed by β -galactosidase to yield o-nitrophenol, that absorbs at 420 nm. A standard curve was plotted, with aggregated human γ -globulins used as a positive control. All reagents were provided in a ready-to-use, commercially available kit by Farmitalia-Carlo Erba, Milano, Italy. Results were expressed as the percentage of inhibition of the probe complex binding to conglutinin. As indicated elsewhere (Ghemmi E, Markovina S. In press), percentages higher than 44 were considered to be positive for circulating immune complexes.

Complement. The levels of C3 and C4 in the serawere measured by radial immunodiffusion on commercial plates (Partigen Behringwerke). For the determination of levels of C3d in the plasma, blood was collected in siliconized plastic tubes that contained EDTA, 0.02 mmol/L. Plasma levels of C3d were measured in PEG (24%)—precipitated plasma on radial immunodiffusion plates prepared using a rabbit anti-human C3d serum (Organon Tecknike) diluted 1:20 in agar (1.5%, w/v, in Michaelis buffer, pH 8.2).

Other parameters. Serum levels of IgG, IgM, and IgA were measured by radial immunodiffusion on commercial plates (Partigen Behringwerke).

Statistical analysis. Statistical evaluations were made by Student's t test, and by calculating the correlation coefficients.

Results

Immunologic studies

Immunohistologic techniques on polymorphonuclear leuhocytes and platelets. The percentages of polymorphonuclear leukocytes and platelets with membrane deposits of immunoglobulins and C3 in groups I, II, and III are reported in Table II. In patients with pre-

^{*}Group I versus group II.

[†]Group II versus group III.

[‡]Group I versus group III.

% Platelets with immunoglobulins and C3 deposits				C1q INH test	KIE test
IgG	IgA	IgM	C3	(%)	(%)
0.3 ± 0.1	0.0	0.3 ± 0.1	0.0	7.6 ± 2.4	57.0 ± 3.9
0.9 ± 0.4 NS* 18.2 ± 2.8 p < 0.001† p < 0.001‡	0.3 ± 0.3 NS* 5.3 ± 0.9 p < $0.001\dagger$ p < $0.001\ddagger$	0.9 ± 0.4 NS* 8.2 ± 1.6 p < 0.001† p < 0.001‡	0.1 ± 0.1 $NS*$ 3.3 ± 0.8 $p < 0.01\dagger$ $p < 0.001\pm$	28.0 ± 4.8 p < 0.001* 76.0 ± 3.4 p < 0.001† p < 0.001‡	73.0 ± 3.3 p < 0.01* 58.0 ± 3.7 p < 0.01† NS‡

eclampsia (group III), the percentages of polymorphonuclear leukocytes and platelets positive for membranebound IgG, IgM, IgA, and C3 were significantly higher than the percentages in normal nonpregnant women and normal pregnant women (groups I and II, respectively). In group III, there also was a significant reduction in the percentage of phagocytic polymorphonuclear leukocytes as compared to the percentages in groups I and II. In addition, in group III, the percentages of platelets with immunoglobulins and C3 deposits were higher than the percentages of polymorphonuclear leukocytes. The differences between groups I and II were not statistically significant. An inverse correlation (p < 0.01) was observed between the occurrence of membrane-bound immunoglobulins and the degree of phagocytosis of C3b-BYP, thus indicating a reduction in free receptors on the surfaces of polymorphonuclear leukocytes obtained from group III. With the exception of platelet-bound IgM, all of the other immunoglobulins and C3 associated with both polymorphonuclear leukocytes and platelets, as well as polymorphonuclear leukocyte free receptors, did not correlate with the severity of preeclampsia. As shown in Fig. 1, 44.7% and 73.7% of patients in group III had positive immunohistologic tests on polymorphonuclear leukocytes and platelets, respectively; the incidence of positive tests in group I (6.6% and 0.0%, respectively) and in group II (0.0% and 10%) in both tests was very

C1q latex agglutination inhibition (C1q INH) test. The inhibitory activity toward Clq was significantly higher in the sera from group III than in those from group II. However, no correlation could be seen with the severity of preeclampsia. The Clq inhibitory activity in group II was also significantly higher than that in group I (Table II).

Conglutinin immunoenzymatic (KIE) test. The inhibition of the binding of β-galactosidase: anti-β-galactosidase complex to bovine conglutinin was significantly higher in the sera obtained from patients in group II than in the sera from group III. No difference was found between groups III and I (Table II).

Complement and immunoglobulins. The serum

levels of C3 and C4 were significantly increased in group II (mean \pm SEM, 116.0 \pm 4.0 and 35.0 \pm 1.5, respectively; p < 0.001) and group III (118.0 \pm 4.3, p < 0.001 and 32.0 \pm 2.1, p < 0.05) when compared to group I (88.0 \pm 2.3 and 26.5 \pm 1.2). Although no differences could be found in the levels of C3d among groups III (6.0 \pm 0.6), II (4.0 \pm 0.8), and I (4.3 \pm 0.4), a positive correlation was observed between the levels of C3d (range, 2.0 to 12.5 mg/100 ml) and the severity of preeclampsia.

No differences between group III and groups I and II were found in regard to the levels of immunoglobulins.

Comment

The results of this study demonstrate the occurrence of circulating immune complexes in preeclampsia. Significant increases in the interactions in vivo between immunoglobulins and complement C3 with polymorphonuclear leukocytes and platelet surfaces were also found in patients with preeclampsia (group III) in comparison to normal pregnant (group II) and normal nonpregnant women (group I). The results obtained with the Clq INH test but not with the KIE test suggest a similar pattern in the three groups of subjects.

Previous reports from our laboratory showed the presence of high levels of immunoglobulins and C3 bound to polymorphonuclear leukocytes during the active phases of diseases mediated by circulating immune complexes, thus suggesting an interaction in vivo between circulating immune complexes and the cell surface receptors for the Fc-fragment of complexed immunoglobulins and the C3b complement fraction.⁶⁻⁸ This hypothesis was further strengthened by the finding of an inverse correlation between the amount of immunoglobulins and C3 on polymorphonuclear leukocyte surfaces and the ability of polymorphonuclear leukocytes to phagocytize opsonized substrates through their receptors for the C3b fraction. Similarly, high levels of immunoglobulins associated to platelets were found in immunopathologic states.13 The stimulation of human platelets, through their surface receptors for the Fc-fragment of complexed immunoglobulins, in-

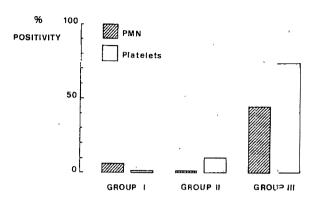


Fig. 1. Incidences of positive immunohistologic tests on polymorphonuclear leukocytes (PMN) and platelets in the hree study groups.

duces their aggregation and the release of endogranular constituents.14

Aggregometric studies on platelets stimulated with ristocetin, that specifically binds the Fc-fragmeric of complexed immunoglobulins, showed an inverse correlation between the response of platelets to ristoctin and the levels of immunoglobulins associated with their surfaces. 15 This favors the hypothesis that these receptors can be saturated in vivo by complexed immunoglobulins. The platelet activation in vivo seems tc be higher in preeclampsia than in normal pregnan y.16 This finding may be correlated with the higher levels of immunoglobulins associated with platelets in vivo found in group III.

The data in the literature are controversial with regard to the occurrence of circulating immune cmplexes in preeclamptic as well as normal pregnarcy. The discrepancy in results has been explained a methodologic basis, such that different assays may or may not detect immune complexes, in relationship to the heterogeneity of the classes, subclasses, sizes, and complement binding capacity of immunoglobules. Our studies in which the Clq INH test was used indicate a significant increase in circulating immune complexes in preeclamptic women (group III), as compared to the findings in normal pregnant (group II) and normal nonpregnant women (group I). However, circulating immune complexes were also present in sign ficant amounts in normal pregnancy (group II). The latter results were confirmed by the KIE test. This is in agreement with the contention that circulating immune complexes occur in normal pregnancy and, therefore, might have a physiologic role. Contrary to what has been observed with the Clq INH test, the levels of circulating immune complexes detected by the KIE test were not increased in patients with preeclamosia (group III). The discrepancy between the resu ts obtained with the CIq INH and the KIE tests might be ascribed to differences in sensitivity and specificary of the assays, as well as to the type of circulating inmune complexes involved. The high positivity for immunoglobulins but not for C3 associated with platelets might indicate that circulating immune complexes present in preeclampsia have a low complement binding capacity. The negative results obtained by the KIE test but not by the CIq INH test are consistent with this hypothesis.

Apparently, no intravascular complement activation occurs in patients with preeclampsia (group III), since the plasma levels of C3 and C4 and those of C3d were not respectively decreased or increased. However, the plasma levels of C3d were markedly increased in the most severe cases of preeclampsia.

In conclusion, the demonstration of the interaction in vivo between circulating immune complexes and the surface receptors of polymorphonuclear leukocytes and platelets may point to a saturation of the capacity of these two cellular systems for clearing circulating immune complexes from the circulation. In addition, receptor-mediated stimulation of polymorphonuclear leukocytes and platelets by circulating immune complexes is known to produce the release of mediators with potent inflammatory activities that might play a role in the pathogenesis of the profound structural and hemodynamic alterations observed in the placenta and kidneys of patients with preeclampsia.

We thank Professor Roberto Corradetti for his helpful advice.

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Disposition of ethanol in maternal blood, fetal blood, and amniotic fluid of third-trimester pregnant ewes

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The disposition of ethanol in maternal arterial blood, fetal arterial blood, and amniotic fluid of nine conscious, cannulated pregnant ewes (128 to 137 days gestation) was determined for 1-hour maternal intravenous infusion of ethanol, 1 gm/kg maternal body weight. The maternal arterial blood and fetal arterial blood etnanol concentration-time curves were virtually superimposable up to 14 hours. The apparent zero-order ethanol elimination rates for maternal arterial blood and fetal arterial blood were similar. There was a time lag in the transfer of ethanol into amniotic fluid relative to fetal arterial blood, and the peak ethanol concentration in amniotic fluid was significantly lower than the concentrations in maternal arterial blood and fetal arterial blood. The apparent zero-order ethanol elimination rate for amniotic fluid was slower, but not significantly so, compared with the ethanol elimination rates for maternal arterial blood and fetal arterial blood. Ethanol-derived acetaldehyde was found in maternal arterial blood, fetal arterial blood, and amriotic fluid at concentrations at least 1003-fold lower than the respective ethanol concentrations. The data indicate that, for administration of this ethanol dosage regimen to the thirdtrimester pregnant ewe, there is rapid, bidirectional placental transfer of ethanol; elimination of ethanol from the letus is regulated primarily by maternal elimination of ethanol; the amniotic fluid may serve as a reservoir for ethanol in utero; and there is appreciable acetaldehyde-metabolizing capacity. (AM J OBSTET GYNECOL 1985;152:583-90.)

Key words: Ethanol, amniotic fluid, fetal and maternal arterial blood, pregnant ewes

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Over the last decade, data from many epidemiologic investigations and experimental animal studies have implicated ethanol as a teratogen. The cluster of central nervous system dysfunction, prenatal and postnatal growth deficiency, and craniofacial abnormalities observed in the offspring of mothers who consumed high doses of ethanol during pregnancy has been referred to as the fetal alcohol syndrome. Recently, it was reported that ingestion of a single, low dose of ethanol by near-term pregnant women resulted in decreased fetal breathing movements for about 3 hours without affecting gross fetal body movements or fetal heart

rate.3 Hence, a wide spectrum of possible effects of ethanol on the fetus would appear to exist.

Although the fetal effects of maternal ingeston of ethanol have been extensively investigated, relatively few studies on the pharmacokinetics of ethanol during pregnancy have been reported. In a recent study of second-trimester pregnant women who ingested a single, low dose of ethanol, differential disposition Eethanol in maternal blood and amniotic fluid was observed.4 This was characterized by slow transfer of ethanol into the amniotic fluid and a slower elimination of ethanol from the amniotic fluid than from the maternal blood. In view of the involvement of the fetus in amniotic fluid dynamics,5 the proposal has been made that the amniotic fluid may act as a reservoir for etharol in utero, so that the fetus is exposed to ethanol for a longer time than would be predicted on the basis of the concentration of ethanol in maternal blood.4

In order to characterize more fully the pharmaco-kinetics of ethanol during pregnancy, we chose the conscious cannulated third-trimester pregnant ewe model. The objectives of this study were to elucidate the disposition of ethanol in maternal and fetal blood and amniotic fluid, and to determine whether acetaldelyde, the pharmacologically active proximate metabolize of ethanol, was present in these biologic fluids. The ethanol dosage regimen, I gm of ethanol per kilogram of maternal body weight given by maternal intravedous infusion over I hour, was selected on the basis of a preliminary study in the pregnant ewe, which demonstrated ethanol-induced suppression of fetal breathing activity, but no change in fetal cardiovascular function.

Material and methods

Nine pregnant ewes of mixed breed were used in the study and were cared for in compliance with the principles of the Canadian Council on Animal Care. At 123 to 132 days' gestation (term, 147 days), anesthesia was induced in each ewe by intravenous administration of 40 mg of sodium thiopental; after intubation, surgical anesthesia was maintained with 1.5% halothane ard a 1:1 mixture of nitrous oxide and oxygen at a flow ≡ate of 1 L/min with the use of closed-circuit ventilation. Under sterile conditions, the uterus was expozed through an abdominal midline incision. The fetus vas partially exteriorized, and polyvinyl catheters were placed in the trachea, brachiocephalic trunk via a fcrelimb artery, sagittal sinus, inferior vena cava via a pelal vein, and amniotic cavity (secured to the shoulder or hindlimb of the fetus). Electrodes of stainless steel, Teflon-coated wire were implanted biparietally on the d□ra for electrocorticographic recording, through the Dr-bital ridge of the zygomatic bone of each eye for fetal electro-oculographic recording, and over the sternem

for fetal heart rate recording. A reference electrode was placed in the loose connective tissue overlying the occipital bone of the skull. The catheters, filled with heparinized saline solution, and the electrodes were exteriorized through the flank of the ewe, and the abdomen was closed in layers. Polyvinyl catheters were then placed in the maternal femoral artery and vein. At the time of operation, 1 gm of dihydrostreptomycin and 800,000 units of penicillin G were administered intramuscularly to the ewe; after the uterus was closed, one million units of penicillin G was injected into the fetal pedal vein and into the amniotic cavity. Administration of antibiotics was continued daily for 3 days. All catheters were flushed daily. The ewes were allowed to recover for 4 or 5 days postoperatively, and were housed individually in cages and provided with food and water ad libitum prior to and during the study. Experimentation was conducted at 128 to 137 days' gestation. Continuous recordings of tracheal pressure, electrocortical activity, electro-ocular activity, arterial blood pressure and heart rate of the fetus, and amniotic fluid pressure were conducted 24 hours prior to, during, and 24 hours after maternal administration of ethanol. These results are reported separately.8

Ethanol (40% v/v, Alcohol, Liquor Control Board of Ontario) was administered in a dose of I gm/kg maternal body weight over I hour into the maternal inferior vena cava by means of a Harvard infusion pump (Harvard Apparatus, Dover, MA). A one-milliliter aliquot each of maternal arterial blood (femoral artery), fetal arterial blood (brachiocephalic artery), and amniotic fluid was collected at selected time intervals prior to ethanol infusion (0 hour), during the 1-hour infusion, and up to 13 hours after the end of infusion (14 hours). All nine pregnant ewes were studied to 6 hours, and three of these animals were further monitored to 14 hours. Because of difficulties in maintaining patency of the catheter in the amniotic cavity, reliable monitoring of the amniotic fluid was only possible in three ewes up to 6 hours and in one ewe up to 14 hours.

Each sample was analyzed quantitatively for ethanol and its proximate metabolite, acetaldehyde, by a modification of an established gas-liquid chromatographic procedure with the use of head-space analysis. The assay was modified to minimize artifactual formation of acetaldehyde from ethanol during preparation of the sample. Within 30 seconds of collection of the sample, 0.1 ml of each sample was placed in an ice-cold 0.9 ml solution that contained 0.865 ml of a saline solution of 34 mg/ml of perchloric acid and 65 µg/ml of sodium azide, 0.01 ml of an aqueous solution of 76 mg/ml of thiourea and 0.025 ml of an aqueous solution of 3 mg/ml of 1-propanol (internal standard of the assay). The sample was mixed for 5 seconds and then centrifuged

at 13,000 × g for 30 seconds in a microcentrifuge (Fisher Model 235A, Fisher Scientific, Toronto, Canada). A 0.2 ml aliquot of the supernatant was placed in a 1.5 ml glass vial, which then was sealed with a silicone septum and immediately frozen on dry ice until analyzed within 36 hours. The accuracy of the procedure was $101.9 \pm 0.7\%$ (SEM) for ethanol and $100.6 \pm 1.3\%$ (SEM) for acetaldehyde, and the within-day coefficient of variation of the assay did not exceed 2.5% for ethanol and 6.2% for acetaldehyde. The lower limit of quantitative sensitivity of the assay was 0.005 mg/ml for ethanol and 0.1 µg/ml for acetaldehyde. If ethanol or acetaldehyde was not measurable in a biologic sample. the analyte concentration was considered to be zero for the purposes of data presentation and analysis. Blank samples of maternal arterial blood, fetal arterial blood, and amniotic fluid were spiked with known concentrations of ethanol and were left for 30 seconds at room temperature. The samples were then analyzed for acetaldehyde. A curve for artifactual formation of acetaldehyde over the ethanol concentration range studied was established for each biologic fluid and was used to correct the acetaldehyde concentration that was measured in each experimental sample. Each sample of biologic fluid was analyzed in duplicate.

Paired samples of fetal arterial and venous blood (2 ml each) were withdrawn simultaneously from the brachiocephalic artery and sagittal sinus at 1 hour prior to ethanol infusion, at the end of I hour of maternal infusion of ethanol, and at 1 hour after ethanol infusion. Each sample of blood was analyzed for pH, PO₂, PCO₂, hemoglobin content, oxygen content, and glucose concentration. These results, together with the data for brain and myocardial blood flow in the fetus prior to, during, and after maternal infusion of ethanol determined by the radiolabeled-microsphere technique, will be reported separately.10

The ethanol and acetaldehyde concentrations and kinetic parameters are expressed as the mean \pm SD. The zero-order ethanol elimination rates for maternal arterial blood, fetal arterial blood, and amniotic fluid were calculated from the apparent linear portion of the elimination phase of the respective ethanol concentration-time curve for each animal by use of linear regression analysis.4 The kinetic parameters, maximum velocity (V_{max}) and Michaelis constant (Km) for in vivo biotransformation of ethanol, were calculated from the maternal arterial blood ethanol concentration-time curve of each of the three animals studied to 14 hours, as previously described.11 Statistical comparison of the ethanol and acetaldehyde concentrations and ethanol elimination rates for maternal arterial blood, fetal arterial blood, and amniotic fluid was conducted by Student's t test for paired data. Two sets of data

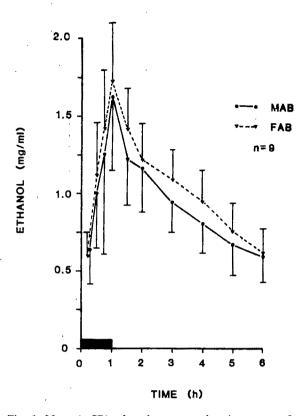


Fig. 1. Mean (±SD) ethanol concentration-time curves for maternal arterial blood (MAB) and fetal arterial blood (FAB) of nine pregnant ewes during and after 1-hour maternal intravenous infusion of ethanol, 1 gm/kg maternal body weight

were considered to be statistically different when $p \le 0.05$.

Results

The mean (±SD) ethanol concentration-time curves for maternal arterial blood and fetal arterial blood of nine pregnant ewes for 1-hour maternal intravenous infusion of ethanol, I gm/kg maternal body weight, are presented in Fig. 1. During infusion, the ethanol concentration was not significantly different between maternal arterial blood and fetal arterial blood, and the peak ethanol concentrations were 1.631 ± 0.480 mg/ ml and 1.738 ± 0.449 mg/ml in maternal arterial blood and fetal arterial blood, respectively, which occurred at the end of the 1-hour infusion. These data indicated rapid placental transfer of ethanol into the fetal systemic circulation during maternal administration of ethanol. The maternal arterial blood and fetal arterial blood ethanol concentrations then declined rapidly during the 1 to 2-hour interval, thus indicating rapid extravascular distribution of ethanol in both the mother and the fetus. During the 2- to 6-hour interval, the ethanol concentration in maternal arterial blood and fetal arterial blood declined more slowly, thus indicat-

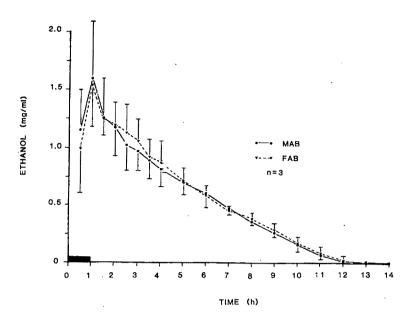


Fig. 2. Mean (\pm SD) ethanol concentration-time curves for maternal arterial blood (MAB) and fetal arterial blood (FAB) of three pregnant exists during and after 1-hour maternal intravenous infusion of ethanol, 1 gm/kg maternal body weight (\blacksquare).

ing that the elimination phase was achieved. The apparent linear decline in ethanol concentration was indicative of zero-order kinetics of elimination. The ethanol elimination rates for maternal arterial blood and fetal arterial blood were calculated for each of the animals; the mean values were 0.123 \pm 0.021 mg \cdot ml $^{-1}$ · hr $^{-1}$ for maternal arterial blood, and 0.141 \pm 0.042 mg \cdot ml $^{-1}$ · hr $^{-1}$ for fetal arterial blood, which were not statistically different.

Three pregnant ewes were studied for 14 hours, and the mean ethanol concentration-time curves for maternal arterial blood and fetal arterial blood are presented in Fig. 2. Apparent zero-order elimination of ethanol in maternal arterial blood and fetal arterial blood occurred up to 11 hours, after which time the ethanol concentration declined nonlinearly and wasvirtually nonmeasurable by 13 hours. The mean mate-nal arterial blood and fetal arterial blood ethanol concentrations were virtually identical throughout the elmination phase. The mean zero-order ethanol elimination rates for these three animals were $0.115 \pm 0.032 \,\mathrm{mg}$ ml-1 · hr-1 for maternal arterial blood and 0.113 ± 0.029 mg · ml⁻¹ · hr⁻¹ for fetal arterial blood, which were not statistically different. The kinetic parameters, V_{max} and Km for in vivo biotransformation of ethanol, were calculated from the individual maternal arterial blood ethanol concentration-time curves of the three ewes, since elimination of ethanol from the maternal compartment has been reported to be the major Factor regulating the elimination of ethanol from the maternal-fetal unit,12 and hepatic biotransformation is

the major route of elimination of ethanol in adult mammalian species. The mean values for V_{max} and Km were 0.135 \pm 0.012 mg·ml⁻¹·hr⁻¹ and 0.073 \pm 0.029 mg/ml ethanol, respectively. The V_{max} was not statistically different from the zero-order ethanol elimination rate for maternal arterial blood.

The ethanol concentration-time curves for maternal arterial blood, fetal arterial blood, and amniotic fluid were determined in three pregnant ewes, and the mean data are presented in Fig. 3. Ethanol was transferred more slowly into amniotic fluid than into fetal arterial blood; ethanol continued to accumulate in amniotic fluid after the 1-hour maternal infusion and reached the peak concentration of 0.920 ± 0.192 mg/ml at 3 hours, which was significantly less (p \leq 0.05) than the peak ethanol concentrations of 1.637 ± 0.512 mg/ml in fetal arterial blood and 1.512 ± 0.352 mg/ml in maternal arterial blood, which occurred at I hour. The amniotic fluid ethanol concentration then declined linearly, indicating apparent zero-order kinetics of elimination. The mean ethanol elimination rate for amniotic fluid was $0.103 \pm 0.015 \text{ mg} \cdot \text{ml}^{-1} \cdot \text{hr}^{-1}$, which was not statistically different from the elimination rates of $0.114 \pm 0.010 \text{ mg} \cdot \text{ml}^{-1} \cdot \text{hr}^{-1}$ for maternal arterial blood and $0.116 \pm 0.014 \text{ mg} \cdot \text{ml}^{-1} \cdot \text{hr}^{-1}$ for fetal arterial blood in these three animals. In one ewe that was studied for 14 hours (Fig. 4), the peak ethanol concentration in amniotic fluid was less than the peaks in both maternal arterial blood and fetal arterial blood and occurred 1.5 hours after the end of the 1-hour maternal infusion of ethanol. However, during the elimination phase of the ethanol concentration-time curves, the concentration of ethanol in amniotic fluid was greater than that in maternal arterial blood and fetal arterial blood, even though the ethanol elimination rate was not apparently different among the three biologic fluids.

Ethanol-derived acetaldehyde was measurable in maternal arterial blood, fetal arterial blood, and amnotic fluid, as demonstrated by the mean data for three pregnant ewes that were studied for 6 hours (Fig. 5). There was appreciable interanimal variability in the acetaldehyde concentration in each of the three biologic fluids, as indicated by the standard deviation values. Also, the acetaldehyde concentration-time curves for maternal arterial blood, fetal arterial blood, and amniotic fluid did not parallel the respective ethanol concentration-time curves (Fig. 3). Statistical analysis (Student's t test for paired data) revealed that the acetaldehyde concentration in maternal arterial blood was significantly greater (p ≤ 0.05) than that in fetal arterial blood over the 1- to 6-hour interval. In those animals studied up to 14 hours, acetaldehyde in maternal arterial blood, fetal arterial blood (three ewes), and amniotic fluid (one ewe) remained at apparent plateau concentrations (shown at 6 hours in Fig. 5) over the 6to 10-hour interval, declined thereafter, and was nonmeasurable by 13 hours.

Comment

The pharmacokinetics of ethanol during the third trimester of pregnancy were elucidated as part of a comprehensive study of the acute effects of maternal administration of ethanol on fetal breathing activity and fetal brain function and metabolism in the conscious, cannulated pregnant ewe. The ethanol dosage regimen, 1 gm/kg maternal body weight given as a 1hour maternal intravenous infusion, was selected to avoid major changes in fetal cardiovascular function, blood gases, and pH. The only significant change was a small increase in fetal heart rate from 162 ± 3 bpm during the 4-hour period prior to maternal infusion of ethanol to 168 ± 3 bpm during the 4-hour period after the initiation of ethanol administration (p ≤ 0.05).8 Thereafter, the fetal heart rate was not statistically cifferent from the control rate.

The maternal arterial blood and fetal arterial blood ethanol concentration-time curves during and up to 13 hours after the 1-hour maternal infusion of ethanol were virtually superimposable (Figs. 1 and 2). These data indicated that there was rapid, bidirectional placental transfer of ethanol between the maternal and fetal compartments, and that the ethanol concentration in maternal arterial blood was predictive of that in fetal arterial blood for these experimental conditions. Ma-

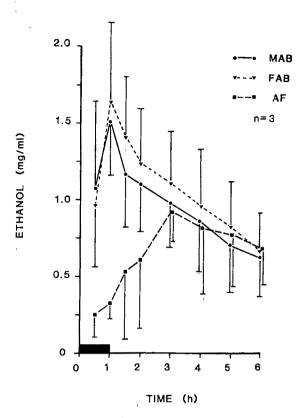


Fig. 3. Mean (±SD) ethanol concentration-time curves for maternal arterial blood (MAB), fetal arterial blood (FAB), and amniotic fluid (AF) of three pregnant ewes during and after 1-hour maternal intravenous infusion of ethanol, I gm/kg maternal body weight (=).

ternal and fetal venous blood ethanol concentrationtime curves have been reported to be similar in conscious, third-trimester pregnant ewes that were given maternal intravenous infusion of ethanol in the dosage regimen of 0.5 gm/kg over 0.5 hour (5-hour study).14 When 3 gm/kg of ethanol was administered by maternal intravenous bolus over 1 to 2 minutes to anesthetized third-trimester pregnant rhesus and cynomolgus monkeys, the ethanol concentration in fetal umbilical venous blood was about one third of that in maternal uterine arterial blood throughout the 1.5-hour study.15 With this ethanol dosage regimen, a transient collapse of the umbilical vasculature and fetal hypoxia and acidosis occurred. Hence, it would appear that rapid maternal administration of a high dose of ethanol can inhibit, at least partly, the placental transfer of ethanol in the maternal-fetal unit, perhaps by decreasing umbilical blood flow.

In this study, the apparent zero-order ethanol elimination rate from maternal blood of the nine pregnant ewes was $0.123 \pm 0.021 \text{ mg} \cdot \text{ml}^{-1} \cdot \text{hr}^{-1}$, which was similar to that reported for pregnant women at term $(0.14 \text{ mg} \cdot \text{ml}^{-1} \cdot \text{hr}^{-1})^{16}$ and pregnant women at 16 to

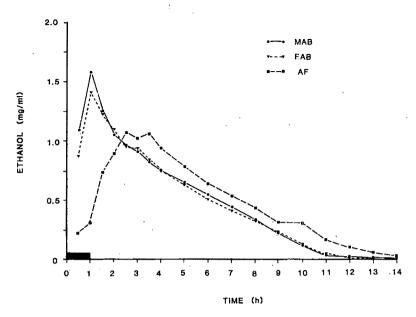


Fig. 4. Ethanol concentration-time curves for maternal arterial blood (MAB), fetal arterial blood (FAB), and amniotic fluid (AF) of one pregnant ewe during and after 1-hour maternal intravenous infusion of ethanol, 1 gm/kg maternal body weight (=).

18 weeks' gestation (0.15 \pm 0.01 mg \cdot ml⁻¹ \cdot hr⁻¹).⁴ Determination of the complete maternal arterial blood ethanol concentration-time curve for three pregnant ewes (Fig. 2) revealed that there was an apparent linear ethanol elimination phase followed by a nonlinear terminal elimination phase. Recently, it was proposed that the complete time course of ethanol elimination from the blood of mammalian species is more adequately described by Michaelis-Menten kinetics.11 Since ethanol elimination in mammalian species occurs primarily by hepatic biotransformation, 15, 17 Michaelis-Menten kinetic analysis provides an estimate of the maximum rate of ethanol biotransformation in vivo (maximum velocity, V_{max}) and the ethanol concentration for one half V_{max} (Michaelis constant, Km), which is an assessment of the affinity of the ethanol-metabolizing system for the substrate, ethanol.11 In the three pregnant ewes, the Km value of 0.073 ± 0.029 mg/ml ethanol was similar to the Km of 1 to 2 mM (0.046 to 0.092 mg/ml) ethanol for adult human liver alcohol dehydrogenase,17 which indicates that this enzyme is primarily involved in regulating the rate of ethanol biotransformation in vivo in the pregnant ewe. Since the apparent zero-order ethanol elimination rates for maternal arterial blood and fetal arterial blood were similar in this study, the elimination of ethanol from the fetal lamb during the third trimester would appear to be regulated primarily by maternal hepatic biotransformation involving the enzyme alcohol dehydrogenase. This postulate is supported, at least in part, by the findings that, in thirdtrimester pregnant rhesus monkeys administered eth-

anol prior to delivery of the fetus, the maternal and fetal blood ethanol elimination rates were similar, whereas the ethanol elimination rate in the neonate was 25% of the maternal elimination rate.¹²

The pharmacokinetics of ethanol in maternal arterial blood, fetal arterial blood, and amniotic fluid of three pregnant ewes demonstrated that the transfer of ethanol into amniotic fluid was slower than that into fetal arterial blood, and the peak amniotic fluid ethanol concentration, which occurred at 3 hours, was lower than the peak ethanol concentration in fetal arterial blood and maternal arterial blood at the end of the 1-hour maternal infusion of ethanol (Fig. 3). The relative time lag and magnitude of maximum ethanol concentration in amniotic fluid compared with maternal arterial blood were similar to the observations made in amniotic fluid and maternal venous blood of second-trimester pregnant women who ingested a single dose of 0.3 gm/kg of ethanol,4 and of third-trimester pregnant ewes that received 0.5 gm/kg of ethanol over 0.5 hours by maternal infusion.14 In the third trimester of pregnancy, the major contribution of fluid into the amniotic cavity is fetal urine.5,6 In view of this and the relative delay in the appearance of ethanol in amniotic fluid compared with fetal arterial blood, fetal urinary excretion would appear to be the major route of transfer of ethanol into the amniotic fluid.

The ethanol elimination rate from amniotic fluid was slower, but not significantly so, than the elimination rate from either maternal arterial blood or fetal arterial blood. In second-trimester pregnant women, the rate of ethanol elimination from amniotic fluid was about half that from maternal venous blood.4 The lack of statistical difference between the maternal arte-ial blood and amniotic fluid ethanol elimination rates in the third-trimester pregnant ewe may be due to the fact that the fetal lamb differs from the human fetus in taat the former is surrounded not only by the amniotic ac but also by the allantoic sac. Hence, the elimination of ethanol from ovine amniotic fluid could involve ciffusion across the amniotic and allantoic membraLes into the allantoic fluid, followed by diffusion across the chorioallantoic membrane into the maternal compatment, in addition to redistribution of ethanol from the amniotic fluid directly into the fetus via fetal swallowing of amniotic fluid and reabsorption of ethanol in the intestine.5 In one pregnant ewe that was studied for 14 hours, the concentration of ethanol in amniotic fluid was higher than the concentrations in maternal artemal blood and fetal arterial blood throughout the 2.5- to 14-hour interval (Fig. 4). This indicated that the amniotic fluid might serve as a reservoir for ethanol in utero, which could result in prolonged exposure of the fetus to ethanol.

Acetaldehyde, the proximate metabolite of ethanol, is pharmacologically active.7 The role of acetaldehyde in the actions of ethanol is controversial,7,17 and E/idence exists that both supports¹⁸ and refutes¹⁹ the proposed involvement of acetaldehyde in the fetal alcohol syndrome. In the present study, acetaldehyde was found in maternal arterial blood, fetal arterial blocd, and amniotic fluid both during and after maternal mtravenous infusion of ethanol, as demonstrated by the data of three pregnant ewes that were studied up to 6 hours (Fig. 5). There was appreciable interanimal variability in the maternal arterial blood, fetal arteral blood, and amniotic fluid acetaldehyde concentrations as indicated by the standard deviation values. The maternal arterial blood acetaldehyde concentration at any given time interval was at least 1000-fold lower than the respective ethanol concentration (Fig. 3), which indicated that only small amounts of acetaldehyde escaped from the acetaldehyde-oxidizing capacity of the maternal liver, which primarily involves aldehyde dehydrogenase in sheep,17,20 and diffused into the maternal systemic circulation. Since the maternal arteral blood acetaldehyde concentration-time curve did not parallel the maternal arterial blood ethanol concentration-time curve, it would appear that the maternal hepatic aldehyde dehydrogenase activity was sufficient o oxidize most of the ethanol-derived acetaldehyde o acetate over a wide range of ethanol concentration. The lower concentration of acetaldehyde in fetal aterial blood compared with that in maternal arteral blood indicated that there was extrahepatic acetald=-

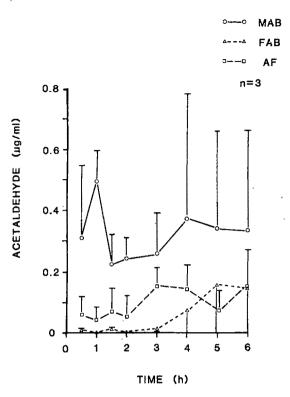


Fig. 5. Mean (±SD) acetaldehyde concentration-time curves for maternal arterial blood (MAB), fetal arterial blood (FAB), and amniotic fluid (AF) of three pregnant ewes during and after 1-hour maternal intravenous infusion of ethanol, 1 gm/ kg maternal body weight.

hyde-oxidizing capacity which regulated the transfer and accumulation of this active metabolite in the fetus. There have been no previous reports on the presence of acetaldehyde in maternal and fetal blood and amniotic fluid of third-trimester pregnant ewes after maternal administration of ethanol. However, it has been reported that acetaldehyde was present in low and variable concentration in maternal venous blood of four of six second-trimester pregnant women after ingestion of ethanol, and acetaldehyde also was found in the amniotic fluid of one of these four subjects.4 In view of these data, the fetal effects of acetaldehyde, at concentrations in maternal arterial blood and fetal arterial blood similar to those of the present study, should be examined.

In conclusion, this investigation demonstrates that, for maternal intravenous infusion of 1 gm/kg of ethanol over 1 hour in the third-trimester pregnant ewe, there is rapid, bidirectional placental transfer of ethanol between the maternal and fetal compartments; elimination of ethanol from the fetus is regulated primarily by maternal hepatic biotransformation of ethanol; the amniotic fluid may serve as a reservoir for ethanol in utero; and there is appreciable acetaldehyde-oxidizing capacity to regulate the exposure of the fetus to the proximate metabolite of ethanol. Future studies should examine the effect of repeated Elministration of ethanol on its pharmacokinetics Euring pregnancy.

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Direct-vision sampling of chorionic villi during extra-amniotic instillation of physiologic saline solution: Effect on intrauterine pressure and fetal heart activity

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After 150 ml of physiologic saline solution had been infused into the extra-amniotic space before first-trimester vacuum aspiration abortion, intrauterine pressure ranged between 16 and 23 mm Hg, thus not more than during Braxton Hicks contractions. At chorionic villi sampling during continuous saline solution infusion, fetal heart activity (beats per 15 seconds decreased temporarily from about 36 to about 33. (AM J OBSTET GYNECOL 1985;152:591-2.)

Key words: Chorion, fetal heart activity, first-trimester pregnancy, intrauterine pressure, prenatal diagnosis

We have found that chorionic villi, uncontaminated with maternal tissue, can be sampled under direct vision during continuous saline solution infusion. We report

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here our results of a study on the effect of such instillation on intrauterine pressure and fetal heart activity.

The clinical material included 42 women in the ninth to thirteenth week of gestation. Six of the women were to undergo elective abortion by vacuum aspiration, and the remaining 36 were cases for prenatal diagnosis. In the six cases for elective abortion, intrauterine pressure was recorded during extra-amniotic instillation of 150 ml of saline solution, performed with the patient under general anesthesia. In the 36 cases for diagnosis, fetal

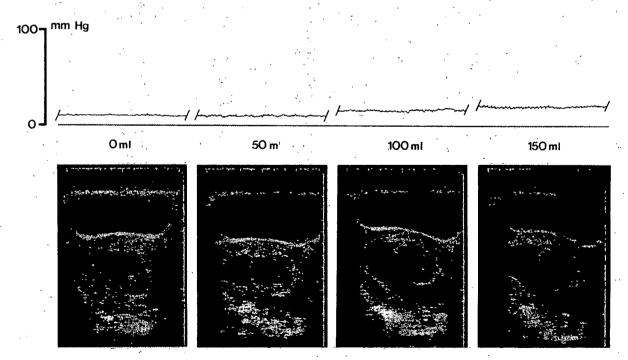


Fig. 1. Intrauterine pressure recording and ultrasound scanning during instillation of 150 ml of physiologic saline solution into the extra-amniotic space of a uterus at 9 weeks' gestation. Note the slight and smooth increase in intrauterine pressure and the absence of any change in amniotic sac outline throughout instillation.

Table I. Fetal heart activity during chorionic villi sampling in 36 cases for prenatal diagnosis

	Fetal heart activity (beats per 15 sec)		
	Range	Mean	-SD
Before sampling During sampling A few minutes after sampling	30-40 25-37 32-41	36.0 33.2 36.8	2.1 2.6 2.2

heart activity was registered during the sampling procedure.

A double catheter system was used, i.e., a IIo. 12 Foley catheter within which a microtransducer catheter 2 mm in diameter (Gaeltec) had been placed. In the external orifice of the Foley catheter a specially constructed tightening plastic plug was inserted, en_rcling the microtransducer catheter. The catheters were introduced, with the Foley catheter balloon lying ust inside the internal cervical orifice. The balloon was distended with 5 ml of fluid. During continuous recording of the intrauterine pressure with a Watanabe Linear Corder, Mark III, 150 ml of saline solution was it stilled extra-amniotically through the Foley catheter by a volumetric infusion pump (Imed, Milton Trading Estate) at a rate of 10 ml/min. The microtransducer catheter was calibrated before and after the recording. The reason for using up to 150 ml of saline solution in this study was our previous finding during samping in women scheduled to undergo vacuum aspiration that the amount of saline solution needed to distend the extra-amniotic space, as calculated from ultrasound scans, was at most 150 ml.

. Before, during, and a few minutes after sampling, fetal heart activity per 15 seconds was counted on real-time ultrasound scans made with a Hitachi EUB-25M linear array or Diasonics DS 20 sector scanner.

Although, with this technique, the extra-amniotic space was distended with saline solution and the instrument inserted into the placenta during continuous infusion, fetal heart activity decreased only slightly (Table I). When sampling was completed and the saline solution allowed to escape, the activity soon returned to presampling values.

Intrauterine pressure increased continuously and smoothly during the extra-amniotic saline solution instillation. Fig. 1 shows an illustrative case. At distention by 150 ml of saline solution, intrauterine pressure ranged between 16 and 23 mm Hg, thus not more than that previously recorded during the physiologic and ordinarily painless contractions of Braxton Hicks.

Sampling of chorionic villi under saline solution instillation seems thus to have only a moderate effect on intrauterine pressure and fetal heart activity. The sampling is now performed without anesthesia or analgesia. It causes little or no discomfort and takes only a few minutes. The woman may return home shortly afterward.

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Vascular reactivity in the hind limb of the pregnant ewe

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The data generated by this study are consistent with the fact that pregnancy and progesterone treatment are associated with an increase in vascular reactivity to phenylephrine and a decrease in reactivity to angiotensin II in the circulation to the hind limb of the ewe. This shows that the way in which vascular reactivity changes during pregnancy is dependent both upon the circulation and the vasoconstrictor being examined. It suggests that sensitivity and contractility could change in opposite directions in this circulation during pregnancy. This will require additional verification, both as to its existence and whether this is an important normal cardiovascular adaptation to pregnancy. (AM J OBSTET GYNECOL 1985;152:593-8.)

Key words: Pregnancy, cardiovascular, vascular reactivity, catecholamine, angiotensin II, sheep

Understanding the manner in which the cardiovascular system adjusts to normal pregnancy has been the subject of research efforts for many years. A particular interest has arisen in how pregnancy may affect the vascular response to vasoactive stimuli, particularly angiotensin II. The observation made was that pregnancy in the human and a number of other species is associated with a decrease in the vascular response to the pressor effects of exogenously administered angiotensin II. In addition, women who develop pregnancy-induced hypertension do not exhibit a similar reduction in responsiveness to angiotensin.^{1,2}

This information generated a number of studies which examined the mechanisms involved in the angiotensin II response observed during pregnancy,² and provided valuable information in regard to this particular systemic pressor response during pregnancy. However, few data are available that describe specific pregnancy-associated responses to vasoactive stimuli in regional circulations other than the circulation for the uterine vascular bed. Since it is the sum of the responses of the regional circulations which constitute pressor responsiveness, this information is the key to an understanding of vascular reactivity during pregnancy.

This study was designed to examine how pregnancy changes the response to vasoactive stimuli in the circulation to the kind limb of the sheep. The hind limb of the sheep is composed primarily of skeletal muscle, the circulation of which is a major determinant of peripheral vascular resistance.³ This fact makes the examination of the circulation to the hind limb during pregnancy potentially valuable in providing information on the normal cardiovascular adaptation to pregnancy.

An in situ hind limb perfusion model was used in the sheep to examine the hind limb pressor responses angiotensin II and phenylephrine (an α -adrenergic agonist) in pregnant and nonpregnant animals. We also examined whether we could duplicate the pregnancy-associated changes in vascular reactivity with short-term progesterone treatment.

Material and methods

Animal model. Fourteen pregnant and 25 nonpregnant mixed-breed ewes were used for this study. Nonpregnant animals were randomly divided into a progesterone-treated group (N = 13) and a nontreated (N = 12) group. These groups were further divided into two sets—one for the angiotensin II study, and the other for the phenylephrine study. In the angiotensin II study, the pregnant ewes (N = 8) ranged in gestational age from 130 to 145 days; in the phenylephrine study (N = 6), they ranged in age from 104 to 142 days. A range of gestational ages was used in the phenylephrine study to determine whether there was a gestational age relationship with the vascular response. Since this was not found, the data have been grouped together. There were two twin gestations in the phenylephrine study, and only singleton gestations in the angiotensin II group.

All ewes were subjected to laparotomy under halothane anesthesia at least 9 days prior to study, with the nonpregnant animals undergoing ovariectomy at the time of laparotomy. Animals were catheterized via saphenous vessels to the level of the abdominal aorta and inferior vena cava. These catheters were used subse-

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Reprint requests: Dr. M. K. McLaughlin, Department of Obstetrics and Gynecology, University of Vermont College of Medicine, Given Medical Building, Burlington, VT 05405. quently for the sampling of blood and the monitoring of blood pressure during the actual experiment. For 3 days prior to the hind limb study, the group designated as progesterone-treated ewes received progesterone in oil (10 mg/kg intramuscularly). This dose we calculated to achieve a plasma level similar to that in the pregnant animals (see Results) for a short time span. Samples of blood were drawn for 3 days prior to and on the day of the experiment for measuring raseline levels of plasma progesterone. In some animals plasma renin activity was measured as well.

Experimental model. The hind limb perfusion technique used in this study was a modification of a uterine perfusion method used previously in sheep as described by Fuller et al. The arterial flow to the hind limb was fixed and isolated, which enabled up to use the perfusion pressure as a direct reflection of the changes in the caliber of the resistance vessels.

Animals were sedated with Nembutal (195 to =60 IU. intravenously) and anesthetized with chloral Tydrate (25 to 40 mg/kg intravenously, Sigma Chemicas). The carotid artery was cannulated with 1/4-inch p=lyvinyl tubing (Tygon Corp.), which was connected to a Varistaltic pump (Manistat Corp.). The femoral arery was isolated, cannulated with polyvinyl tubing, and perfused with the blood drawn from the carotid artery. Heparin (1 mg/kg) was given as a bolus, followed by supplemental doses every 20 to 30 minutes. The flow rate was set in such a way that the hind limb arterial, perfusion pressure was similar to the mean systemic blood pressure. The pump flow rate was calib-ated at the end of each experiment by means of a stopwatch and a graduated cylinder. The pump provided fixed, pulsatile flow to the limb, with the venous dainage proceeding through natural channels. The hind limb arterial pressure was measured either from a eatheter placed distal to the perfusion line via the sarhenous artery or from the perfusion line itself.

Adequacy of isolation was verified by measuing the back pressure with the pump off; this was always between 10 and 20 mm Hg. The quality of the preparation was evaluated by determining the hind limb resistance, blood gases, and blood pH periodically throughout the experiment. The maintenance of homeostasis was assisted by the administration of actated Ringer's solution (15 to 25 ml/min), keeping boxy temperature between 37° and 38° C, maintaining z steady plane of anesthesia, and having the animals breath 100% oxygen through a tracheostomy. By m≡ans of these procedures, the preparations were judged to be stable for at least 3 hours after the initiation of perfusion (see Results). Since venous drainage preceeded through normal venous channels, the animal was able both to oxygenate its own blood and to metabolize the injected drugs. This avoided the need to perform these.

procedures mechanically, so that manipulation of red blood cells was minimized.

Experimental protocol. Subsequent to a 20-minute stabilization period after initiation of the hind limb perfusion, dose-response curves were generated to either phenylephrine (200 to 600 ng/kg/min) or angiotensin II (4 to 20 ng/kg/min). Drugs were infused at a rate of 0.1 to 0.5 ml/min directly into the perfusion line. These doses were chosen to produce a range of vasoconstriction in the limb, but were sufficiently low to be subthreshold in the rest of the systemic circulation (see Results). This was done in order to minimize any baroreflex effects upon the skeletal bed, since the sensitivity of the baroreflex system could change during pregnancy. Each dose was infused randomly and in duplicate for 5 minutes, with at least 10 minutes between the infusions of angiotensin II and 20 minutes between the infusions of phenylephrine. In some of the angiotensin II experiments, the doses were repeated after the administration of prazosin to the hind limb (1 mg). This is a specific α_i -receptor antagonist which was used to determine whether a portion of the pressor response was due to facilitation of endogenous release of norepinephrine and subsequent vasoconstriction. All drugs were diluted in normal saline solution and kept iced until needed.

Data analysis. The change in hind limb perfusion pressure (arteriovenous pressure) that occurred with each dose of drug was determined by subtracting the mean steady-state response at 3 to 5 minutes of drug infusion from the mean perfusion pressure measured during the 3 minutes just prior to drug infusion. These mean values were obtained by visually averaging the millivolt output of the recorder for the time span specified. The hind limb perfusion pressure responses were meaned for each group. The dose-response curves for phenylephrine and angiotensin II were compared within and between groups by means of a two-way analysis of variance with repeated measures and orthogonal contrasts. The orthogonal contrasts portion of this statistical package tests for the linearity of the data points. Any one group was compared to another by means of the Scheffe test for multiple comparisons.6 Other baseline data were compared by analysis of variance.

Biophysical measurements. Blood pressure was continuously monitored by means of Ailtech (Eaton Corp.) pressure transducers; heart rate was derived from the pulse pressure waveform with use of a Gould Biotech, and all measurements were recorded on a rectilinear recorder (Gould Instruments, Cleveland, Ohio). Blood gases were analyzed on a Radiometer blood gas analyzer. Plasma progesterone levels were determined by standard radioimmunoassay procedures. Plasma renin activity was calculated by measuring the generation of angiotensin I with use of the New England Nuclear kit.

Table I. Baseline parameters* for the treatment groups

	Weight (kg)	$\begin{array}{c} Limb \ Q \\ (ml \ min^{-1}) \end{array}$	Mean arterial pressure (mm Hg)	Heart rate (bpm)	Progesterone (ng ml ⁻¹)
Castrate	45 ± 3	129 ± 5	114 ± 4	135 ± 5	0.32 ± 0.04
Progesterone-treated	44 ± 2	116 ± 9	117 ± 4	128 ± 7	39.2 ± 5.0
Pregnant	50 ± 2	169 ± 19*	112 ± 4	126 ± 4	32.3 ± 7.0

Data are the mean = SE.

Results

Baseline parameters. Depicted in Table I are the means in body weight, hind limb blood flow, baseline systemic blood pressure, and heart rate and the plasma progesterone levels for each group. There were no statistically significant differences in body weight between the three groups. The fact that the pregnant ewes were not heavier than the nonpregnant ewes was probably due to the mixed-breed composition of the animals. The flow to the hind limb of the pregnant ewes was significantly greater (p < 0.05) than that measured in the progesterone-treated ewes but was not different (p < 0.10) from that in the nonpregnant animals. Blood pressure values and heart rates between groups were similar and the progesterone levels showed the expected increase over those in ewes subjected to cvariectomy. The progesterone levels in the steroid-treated ewes were not significantly different from those in the pregnant ewes.

The hind limb vascular resistances were calculated at the beginning, middle, and end of each experiment as the limb perfusion pressure (arteriovenous pressure) divided by flow. These values are shown in Fig. 1. and the blood gases and blood pH values are shown in Fig. 2.

There were no significant differences over the time course of the experiment between the initial baseline resistances and the final resistances. The progesterone-treated animals had significantly higher initial resistances than those of the other two groups of animals (p < 0.01). The p value was <0.06 for the comparison of the resistances between the pregnant ewes and the ewes subjected to ovariectomy.

There were no significant differences in the blood gas and blood pH values over the course of the experiment

Experiment I: Dose response to phenylephrine. The dose responses of the changes in hind limb perfusion pressure caused by the administration of phenylephrine are shown in Fig. 3. The dose-response curves were linear (p < 0.001) over the range of doses studied and the groups were significantly different (p < 0.01) from one another. The changes in perfusion pressure with increasing doses of phenylephrine ranged from 22 to 43 mm Hg in the animals subjected to ovariectomy.

Pregnancy markedly increased the sensitivity of the circulation to the hind limb, and shifted the curve to the left of that for the ewes subjected to ovariectomy. However, the slope of the curve for the pregnant animals was significantly less (p < 0.02) than that for the ewes subjected to ovariectomy. Progesterone treatment also increased the sensitivity to phenylephrine, as indicated by the left-hand shift of the dose-response curve. The slope of this dose-response curve was increased when compared to those in the other two groups of animals (p < 0.01). In three of the pregnant animals, lower doses of phenylephrine were administered to produce a smaller range of changes in pressure. In these three animals, the dose-response relationship remained linear with their original curve over a change in perfusion pressure from 10 to 40 mm Hg.

Changes in baseline heart rate, as well as increases in systemic blood pressure, were used as an index of whether the dose of drug being administered was low enough to avoid any significant baroreceptor activity. There were no significant changes in either of these parameters which were associated with either drug infusion.

Experiment II: Dose response to angiotensin II. The dose responses of the changes in hind limb perfusion pressure caused by the administration of angiotensin II are shown in Fig. 4. The dose-response curves were linear (p < 0.001) over the range of doses studied and were significantly different from one another (p < 0.02). The pregnant ewes were less sensitive to the effects of antiotensin II than were the ewes subjected to ovariectomy. Progesterone treatment shifted the curve to the right in such a way that this group of animals had a response which was similar to and not statistically different from that of the pregnant ewes.

In the three nonpregnant, three progesteronetreated, and six pregnant ewes studied, blocking the α_1 -receptor with prazosin did not affect the response to exogenously administered angiotensin II, thus indicating that facilitation of the release of norepinephrine was not a variable between groups.

Comment

The purpose of this study was to examine how pregnancy and progesterone treatment affected vascular

^{*}p < 0.05 versus progesterone-treated.

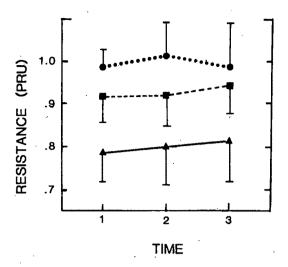


Fig. 1. The calculated hind limb vascular resistance (ram Hg min m1⁻¹) in the three groups of ewes at the beginnin;, middle, and end of the experiments. Data are the mean ± SD. Pregnant ewes (**A**), castrate ewes (**B**), progesterone-**B** eated ewes (**O**).

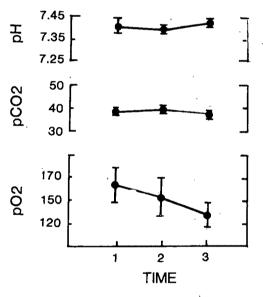


Fig. 2. The arterial blood gases and pH in all animals (n = 39) at the beginning, middle, and end of the experiments. Data are the mean \pm SE.

reactivity in the circulation to the hind limb. This circulation was chosen for investigation because the hind limb is composed primarily of skeletal muscle tissue. Because of the mass of skeletal muscle in the body its vascular tone is a major determinant of total peripheral resistance. The second reason for this choice was the fact that this circulation is relatively easy to use in a perfusion experiment because the blood flow is primarily isolated to one large artery.

Two different vasoactive drugs were used in this study. Our purpose was not to compare the actions of angiotensin II to those of phenylephrine, but rather to

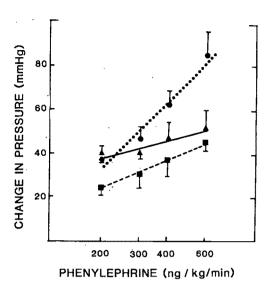


Fig. 3. Semilogarithmic plot of the change in hind limb pressure in response to four doses of phenylephrine. Data are the mean ± SD. Castrate ewes (■), progesterone-treated (●), pregnant (▲).

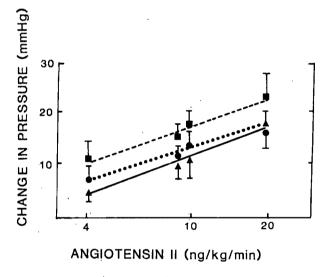


Fig. 4. Semilogarithmic plot of change in the hind limb pressure in response to four doses of angiotensin II. Data are the mean ± SD. Castrate ewes (■), progesterone-treated ewes (●), pregnant ewes (▲).

examine how pregnancy affects vascular reactivity to each of these drugs. Over the dose ranges studied, pregnancy and progesterone treatment were associated with a loss of vascular reactivity to angiotensin II and an increase in vascular reactivity to phenylephrine. For a direct comparison to have been made between the drugs, the protocols should have been designed to achieve the same changes in pressure with the two drugs. However, most of the phenylephrine protocol was completed before the angiotensin II study was begun. In the phenylephrine study, we were able to achieve a good range of changes in perfusion pressure

to the hind limb prior to the drug causing an increase in the systemic blood pressure. An amount of drug which caused an observable increase in systemic blood pressure was our criterion for determining the maximum dose to use. When the angiotensin II study was begun, the highest drug dose which fit this criterion caused a much smaller change in perfusion pressure to the hind limb. Therefore, we were not able to obtain comparable changes in pressure between the two drugs. In the three animals in the phenylephrine protocol in which the lower doses were examined, the dose-response curves remained linear. This suggests, but does not show definitely, that pregnancy is associated with an increase in vascular reactivity in this circulation to the pressor effects of phenylephrine at a range of changes in perfusion pressure that are associated with a reduction in responsiveness to angiotensin II.

A more important observation which can be made from the data in the angiotensin II study is the fact that very little change could be observed in the hind limb vascular resistance prior to the drug dose causing an increase in systemic vascular resistance. This incicates that, when angiotensin II is causing a pressor response in the systemic circulation, the participation of the skeletal muscle circulation to this response must be relatively small compared to other regional circulations. It has been shown in the nonpregnant condition that the skeletal muscle vasculature is relatively insensitive to stimulation by angiotensin II.8 The pregnancyassociated loss of the angiotensin II pressor response which has been observed in the sheep is most likely due to an effect of pregnancy on other major regional circulations, such as the renal and splanchnic circulations.

A number of mechanisms could function to alter angiotensin II reactivity during pregnancy, and most of these have been discussed in the present literature, i.e., the modulation of angiotensin II vasoconstriction through vasodilatory prostaglandins and/or progesterone metabolites, 1,2 or a change in the properties of the angiotensin II receptor population itself.9 What the antual mechanisms are still requires further in vitro analysis.

The fact that progesterone pretreatment caused a reduction in angictensin II sensitivity in this study substantiates previous data obtained in the human and rat. It cannot be ascertained, however, whether the effects of progesterone are direct ones of the steroid upon the vascular smooth muscle or whether they are secondary to other alterations caused by the hormone. For example, progesterone may increase circulating levels of angiotensin II, as happens in pregnancy, with a subsequent increase in the negative feedback mechanism operating on the kinetics of receptor turnover. We did measure plasma renin activity in this study group as an index of the levels of angiotensin II. Although the mean

levels for the pregnant and progesterone-treated animals and those subjected to ovariectomy were 0.87, 0.90, and 0.54 ng/ml/hr, respectively, these values were not significantly different from one another. Other studies have shown a definite increase in plasma renin activity during progesterone treatment. Our small sample size is the most likely cause of the lack of statistical significance.

Both pregnancy and progesterone treatment were observed to increase the sensitivity of the vasculature of the hind limb to the α -adrenergic stimulation by phenylephrine. This change in reactivity could have been due either to a decrease in degradation or to an increase in postjunctional sensitivity.¹⁰ This study was not designed to differentiate between these two possibilities.

Since there is a difference in drug actions between epinephrine, norepinephrine, and phenylephrine, 10 we cannot easily compare our observations with those made in other studies. Moreover, the data which are presently available and recently reviewed1, 11 are conflicting. Pressor responses to epinephrine have been shown to be either unchanged or reduced in the human and reduced in the rat during pregnancy. Norepinephrine pressor activity has been studied in a number of ways in humans, with reports of increases, decreases, or no change in senstivity during normal pregnancy. In the sheep, there is no difference between pregnant and nonpregnant animals in the in vitro response of the calcaneal artery to norepinephrine, but the phenylephrine response was not studied.12 In the perfused hind limb of the rabbit, there is a greater increase in hind limb vascular resistance in response to low-frequency sympathetic stimulation in the pregnant than in the nonpregnant animals when studied under conditions of constant pressure. This difference was lost when the hind limb was restimulated under conditions of constant flow. This discrepancy may be attributable to different pressure-flow curves in the two groups of animals.18 The sheep hind limb in the study reported here was examined by means of a constant-flow condition.

The data from previous work that dealt with the effects of progestational hormones upon the vascular reactivity to catecholamines are unclear. Some studies show an enhanced response to certain amines, whereas other studies show no change.¹ These observed differences do not appear to be species related and do not allow for any definitive conclusions. As indicated above, the effects of progesterone upon the adrenergic sensitivity to phenylephrine could be through direct or indirect effects upon the vascular smooth muscle. Progesterone receptors have been identified in the aortic smooth muscle of the baboon subjected to ovariectomy, although their numbers were thought to be lower than

what would be present in an estrogenic state.¹⁴ It is not known what the progesterone-mediated receptor response might be in vascular smooth muscle. Indirectly, both the pregnant and progesterone-treated animals may have had elevated levels of deoxycorticosterone. Although deoxycorticosterone of itself is not hypertensinogenic in the sheep, it could potentially influence adrenergic sensitivity.^{15, 16}

The concept of vascular reactivity as used in this study includes both the sensitivity and the contractility of the vasculature. An increase in sensitivity to agonist stimulation is demonstrated by a left-hand shift of the dose-response curve, whereas changes in contractility are represented by alterations in the slope of the line. At the range of pressure responses which were examined in the phenylephrine study, the increase in vascular sensitivity during pregnancy was accompanied by a decrease in contractility. A more definitive in vitro study of vessel mechanics will be required before a conclusive statement can be made that vessel contractility is reduced during pregnancy. Collagen and elastin content has been shown to be altered in larger arteries by pregnancy,1 which is probably of sufficient magnitude to alter the stress-strain characteristics of the vessels.¹⁷ The short-term progesterone treatment duplicated the adrenergic sensitivity changes associated with pregnancy, but actually increased contractility, perhaps because of shifts in fluid within the vessel wall.1 This indicates that the chronic effects that pregnancy has upon the vasculature is a complex interplay of direct steroidal effects and multiple other factors (i.e., increased volume load, change in blood pressure, etc.) which cannot be duplicated by short-term administration of steroids.

In summary, these data are consistent with the fact that pregnancy and progesterone treatment are associated with an increase in vascular reactivity to phenylephrine and a decrease in reactivity to angiotensin II in the circulation to the hind limb of the ewe. This shows that the way in which vascular reactivity changes during pregnancy is dependent upon both the circulation and the vasoconstrictor being examined. It suggests that sensitivity and contractility could change in opposite directions in this circulation during pregnancy. This will require additional verification, as to both its existence and whether or not this is an important normal cardiovascular adaptation to pregnancy.

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CURRENT INVESTIGATION

³H-dopamine clearance from the intravascular compartment of the maternal and fetal rhesus monkey

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The clearance of epinephrine and norepinephrine from the intravascular compartment has previously been demonstrated in both pregnant and nonpregnant models. With the use of the pregnant rhesus monkey, this study describes the clearance of dopamine from the fetal and maternal intravascular compartments. In both, the dopamine clearance was biphasic with an initial half-life of 1.5 minutes in fetal blood and 1.0 minute in maternal blood. These studies also confirmed that dopamine does not significantly cross the placenta in an intact form. These observations are consistent with those made for the other two major catecholamines. (AM J OBSTET GYNECOL 1985;152:599-601.)

Key words: Dopamine, intravascular compartment, pregnancy, half-life, rhesus monkey

Previous published reports1. 2 have described the clearance of catecholamines, specifically norepinephrine and epinephrine, from the intravascular compartment in both pregnant and nonpregnant mammals, including the human. Such studies have described a biphasic clearance pattern with an initial catecholamine half-life of I to 4 minutes. Recent studies have confirmed the significance of dopamine as one of the major catecholamines, with significant concentrations in both maternal and fetal plasma. The in vivo metabolism of dopamine in a nonpregnant animal model has been described3; however, to date no clearance studies of dopamine during pregnancy have been reported. This study was undertaken to describe the pattern of radiolabeled dopamine clearance from the intravascular compartment in the maternal and fetal rhesus monkey near term.

Material and methods

Radiolabeled dopamine injection studies were performed in five pregnant rhesus monkeys and their fetusus at 118, 141, 152, 155, and 157 days of gestation. In order to provide access to the intravascular com-

partment, maternal femoral artery and fetal neck vessels (jugular vein in four and carotid artery in one) were catheterized with silicone rubber catheters by sterile surgical technique under ketamine anesthesia (10 mg/ kg intramuscularly).4 At the completion of the fetal vessel catheterization, the catheters were exteriorized, the hysterotomy incision closed, and the animal given a 20- to 30-minute recovery period on its side. At time zero an average 5.2 µCi of 3H-dopamine was injected into the fetal circulation; then 1 ml of samples were withdrawn at 1, 2, 5, 10, and 30 minutes along with sampling of maternal blood at 5, 10, and 30 minutes after fetal injection. In order to prevent fetal hypovolemia during these studies, fetal blood was replaced by an equal volume of unlabeled maternal blood after each sampling.

After the completion of the fetal studies, an average 7.0 μCi of ¹⁴C-dopamine was injected into the maternal circulation; then 1 ml of maternal blood samples were obtained at 1, 2, 5, 10, and 30 minutes after maternal injection. At the completion of these experiments the animals were delivered by repeat hysterotomy; fetal well-being was confirmed by delivery of live-born rhesus infants (all of whom survived, except the markedly immature one at 118 days of gestation).

All of the blood samples were placed in iced tubes containing 1.2 mg reduced glutathione and 1.8 mg of ethyleneglycol-bis (β -aminoethyl ether) N, N¹-tetraacetic acid (EGTA); the plasma was subsequently isolated and stored at -20° C until assayed. Free radiolabeled dopamine was determined by alumina extrac-

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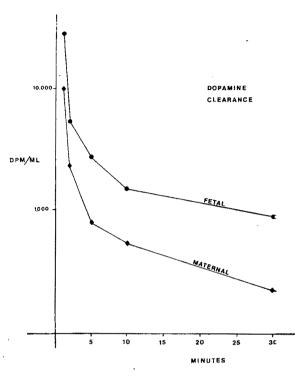


Fig. 1. Mean values reported as disintegrations per m nute per milliliter for radiolabeled dopamine after fetal anc maternal intravascular injections. §H-dopamine was used for fetal injection studies, and ¹4C-dopamine was used for maternal injection studies.

tion techniques performed in duplicate on 50 Ll of plasma samples. 5 Specifically, the pH of the plasma :amples was increased to 9.0 with 1 ml of tromethamine (Tris) buffer, 40 mg of alumina was added, and it was agitated by Vortex mixer over 10 minutes. After the alumina settled, the supernatant was removed. Th∈ alumina was washed with 3 ml of distilled water three times; then the dopamine was eluted with 1 ml of 4.2N hydrochloric acid. The supernatant was collected and counted in 9 ml of Aquasol (New England Nuclear, Boston). The counts were corrected for reco-ery, quenching, and counter efficiency and then reported as disintegrations per minute per milliliter. Analysis of semilogarithmic plots with linear estimates of the cearance slope was used to estimate the intravascular halflife.

Results

As noted in Table I and Fig. 1, rapid clearance of ³H-dopamine occurred from the intravascular compartment of all five of the fetal monkeys. The estimated initial phase half-life was 1.5 minutes, and the second phase was 17.5 minutes. Maternal samples drawn at 5, 10, and 30 minutes after fetal injection confirmed that no significant amount of free dopamine crossed the placenta in these experiments, as noted in Table I. Fig. 1 and Table II demonstrate a very similar pattern of ¹⁴C-dopamine clearance after maternal intravascular in-

Table I. Clearance of ³H-dopamine from fetal and maternal blood after fetal intravascular injection

Time (min)	Fetal blood (dpm/m!)	Maternal blood (dpm/ml)	No.
1	$29,200 \pm 16,024$		2
2	$5,533 \pm 726$	-	5
5	$2,743 \pm 786$	52 ± 29*	5
10	$1,515 \pm 415$	$23 \pm 10*$	5
30	910 ± 168	$24 \pm 16*$	5

Values ± SEM.

*p ≤ 0.05 by paired t test analysis compared to fetal values.

Table II. Clearance of 14 C-dopamine from maternal blood after maternal intravascular injection (n = 5)

Time (min)	Maternal blood (dpm/ml)	
1	$10,472 \pm 3183$	
2	$2,293 \pm 888$	
5	807 ± 106	
10	547 ± 70	
30	227 ± 68	

Values ± SEM.

jection. The estimated initial phase half-life of dopamine in maternal blood was 1.0 minutes, and the second phase was 14 minutes.

Comment

Saarikoski² and Jones and Robinson¹ have previously demonstrated rapid clearance of epinephrine and norepinephrine from the maternal and fetal circulation. These investigators have likewise demonstrated minimal transplacental passage of these two hormones intact. The present study has now confirmed that dopamine is also rapidly cleared from the maternal and fetal circulation and that no significant placental transfer of this hormone occurs. Norepinephrine studies² have demonstrated that the inhibition of placental transport occurs due to rapid metabolism by placental monoamine oxidase and catechol-0-methyl-transferase. Williams et al.3 have demonstrated that these same enzymes are responsible for the in vivo metabolism of dopamine in the nonpregnant rat; Phillippe and Niloff⁶ have similarly observed that dopamine is metabolized in vitro by these enzymes in chorioamnion tissue. Future studies during pregnancy will be needed to confirm that the intravascular clearance and inhibition of placental transfer of dopamine likewise occur because of the activity of these enzymes.

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Clearance of ³H-dopamine from the amniotic fluid in the rhesus monkey

Mark Phillippe, M.D., Susan Haas, M.D., Stephen Evans, M.D., and Prabhat Sehgal, A.M. Boston, Massachusetts

Previous studies have suggested that dopamine is the major catecholamine in the amniotic fluid; however, there are few data available concerning the metabolism of this hormone in the amniotic fluid compartment. With the use of acute ³H-dopamine injection studies into the amniotic sac of pregnant rhesus monkeys, the dopamine half-life was observed to be 29 minutes, the amniotic fluid volume was 113 ml, the metabolic clearance rate was 164 ml/hr, and the calculated production rate was 436 ng/hr. The biphasic pattern of dopamine clearance from this compartment suggests that it is cleared from the amniotic sac by mechanisms similar to those used in the intravascular compartment. (AM J OBSTET GYNECOL 1985;152:601-2.)

Key words: Dopamine, amniotic fluid, pregnancy, metabolic clearance rate, half-life, rhesus monkey

A previous study¹ demonstrated that dopamine is the predominant catecholamine in the amniotic fluid compartment. Measurements of amniotic fluid dopamine suggest that the concentration of this hormone peaks during the antepartum period. Another report² also demonstrated the presence of dopamine metabolites in the amniotic fluid; however, these were static measurements with no indication of their origin or turnover. The metabolic clearance, metabolic fate, and half-ife of dopamine in the amniotic compartment have yet to be established. This study was undertaken to evaluate these parameters in the primate pregnancy.

Material and methods

Under ketamine sedation (10 mg/kg intramuscularly), nine studies were performed on five pregnant rhesus monkeys between 100 to 148 days of gestation.

With utilization of ultrasound guidance (ADR model 2130 with 3.5 MHz linear-array transducer) the amniotic sac was percutaneously catheterized under sterile conditions to allow free access to the amniotic fluid. At the completion of the studies, the catheters were removed and the pregnancies were allowed to continue. An initial 1 ml aliquot of fluid was removed for catecholamine determinations; then 4 µCi of ⁸H-dopamine was injected into the amniotic fluid at time zero. Over the next 50 seconds the amniotic contents were vigorously shaken to allow complete diffusion of the dopamine. At 1, 5, 10, 30, and 60 minutes after dopamine injection, 1 ml aliquots of amniotic fluid were collected for labeled-hormone determinations. Amniotic fluid was replaced by an equal volume of normal saline solution, and the uterine contents were shaken after each sampling. In four of these experiments the volume of amniotic fluid was estimated by dye dilution techniques with use of p-aminohippurate sodium. Endogenous catecholamines were determined by radioenzyme assay techniques (Cat-a-kit, Upjohn Diagnostic).1 Tritium-labeled dopamine was determined by alumina extraction techniques with subtraction of counts due to dihydroxyphenylethanol and dihydroxyphenylacetic acid metabolites (these metabolite counts were determined by

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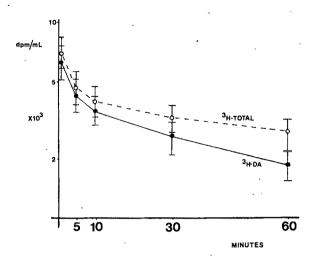


Fig. 1 Mean ± SEM values for total tritium and ³H-dopamine in amniotic fluid at timed intervals after intraamniotic injection of labeled dopamine. Values corrected for recovery, quenching, and counter efficiency are reported as disintegrations per minute per milliliter (dpm/ml).

ethyl acetate extraction techniques).^{3, 4} Volume of distribution, metabolic clearance rate, and amniotic fluid dopamine half-life were calculated by curve peel techniques applied to the mean dopamine disappearance curve.⁵

Results

As noted in Fig. 1, the disappearance curve of 3H -dopamine demonstrated a biphasic clearance pattern with an initial phase estimated at 11.7 minutes and second phase at 56.6 minutes. The amniotic fluid volume measured by the p-aminohippurate sodium dilution technique in four experiments was 104 ± 14 ml (\pm SEM); another estimate of amniotic fluid volume based on the dilution of the 3H -dopamine at 1 minute in the nine experiments gave a value of 122.5 ± 23.9 ml. Utilizing the curve peel technique the volume was calculated to be 113 ml, the metabolic clearance rate was 164 ml/hr, and the overall half-life of dopamine in the amniotic compartment was 29 minutes. The endogenous catecholamine concentrations in these experiments were dopamine = 2660 ± 389 pg/ml

(±5EM, n = 7), norepinephrine = 605 ± 177 pg/ml (n = 8), and epinephrine = 159 ± 33 pg/ml (n = 8). Utilizing the calculated metabolic clearance rate and the mean endogenous dopamine concentration, an estimated production rate for dopamine in these experiments was 436 ng/hr.

Comment

These studies suggest that dopamine is cleared from the amniotic fluid compartment in a biphasic pattern sim lar to the pattern described for the clearance of cateholamines from the intravascular compartment.6 In the amniotic compartment, however, the clearance of copamine is slower, that is, the initial phase half-life is more than two to three times the value described for the intravascular compartment. It has been suggested that the biphasic pattern of catecholamine clearance from the blood occurs because of an initial rapid cellular uptake of these hormones, followed by a slower clearance by hormone metabolism. The observation of increasing concentrations of dopamine metabolites in the amniotic fluid at 60 minutes (indicated by the difference between 3H-dopamine counts and total tritium counts on Fig. 1) and the biphasic clearance pattern imply the same mechanisms may be occurring. However further studies are necessary to evaluate the actual mechanisms responsible for dopamine clearance from the amniotic fluid compartment.

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Diabetes and spontaneous abortion

To the Editors:

The claim recently made in your pages (Miodovnik M, Lavin JP, Knowles HC, et al. AM J OBSTET GYNECOL 1984;150:372-376) that insulin-dependent diabetes was associated with a spontaneous abortion rate substantially higher than occurs in the general population may have been based on flawed procedures and analyses. Any one of the following biases and unacknowledged considerations could have created such an illusion.

- 1. Since it was not explicitly stated that the 91 women forming the study group constituted all the women engaged in the "long-term study of juvenile diabetes" at the University of Cincinnati Medical Center who became pregnant during the period surveyed, it is possible that the 91 women were a self-selected fraction of this whole number who volunteered for the study because of a special situation, for example, as the authors reported, their previous excessive spontaneous abortion rate.
- 2. In distinction to other studies of spontaneous abortion in diabetic pregnancy, in which rates found were not significantly different from that in the general population (see Miodovnik et al., 1984, for references), the Miodovnik survey was prospective and begun early in pregnancy, a procedure that usually reveals a far larger spontaneous abortion rate than retrospective ones do.¹
- 3. The authors did not report the racial composition of their study group, but earlier it was noted² that over 75% of the diabetic pregnancies in their center were black. This is relevant, since it has been found^{3, 4} that nonwhite women have an intrauterine death rate, at all periods of gestation, over twice that of white women.

A great many maternal attributes are known to be associated with spontaneous abortion.^{1, 5} Although it may be difficult to obtain a control group comparable to the study population in all or most of these characteristics, an inquiry into this controversial subject will be unconvincing if it makes no attempt at all to do so. *H. Kalter, Fh.D.*

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Reply

To the Editors:

We would like to thank Dr. Kalter for his interest in our study and address each of his comments.

- 1. As stated in the manuscript, all women in the long-term study were seen by Dr. Knowles at least twice yearly and, as part of long-term overall management, had been instructed to report immediately any menstrual aberration or possibility of pregnancy. Between June 1, 1978, and January 31, 1983, 91 of the 258 women followed in the long-term study had clinically apparent pregnancies: The 91 women enrolled in the present study were followed by Dr. Knowles for an average of 6 years, ranging from 1 to 19 years. The patients were not "a self-selected fraction" of the women in the long-term study of juvenile diabetes as was suggested. Furthermore, other women referred for care of pregnancy and diabetes to Dr. Knowles were not included in the study.
- 2. The present study revealed a higher spontaneous abortion rate than the rate reported in retrospective studies of insulin-dependent diabetic women. Although prospective study will reveal a higher rate of spontaneous abortion, this type of study will more accurately reflect the true incidence of spontaneous abortion.¹ Comparison made in the manuscript to spontaneous abortion rates in the nondiabetic population referred only to clinically apparent spontaneous abortions in prospective studies.²-4
- 3. The racial distribution of the patients in the present study is 70 (77%) white women and 21 (23%) non-white.

We think that our manuscript provides valuable information in regard to the incidence of spontaneous abortion rate among insulin-dependent diabetic women. Furthermore, we feel that differences in results of this study and previously reported studies are not due to methodologic errors of this study.

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Prenatal screening for hemoglobinopathies

To the Editors:

The recent article on screening for hemoglot-inopathies by Stein et al. (Am J OBSTET GYNECOL 1984; 150:333) could be useful to those who wish to et up similar screening programs.

I would like to call to your attention the failure of the protocol to adequately address the identification of individuals with sickle cell—thalassemia of the β^- variety. On electrophoresis, these individuals will have both an S and an A band. Quantification will reveal that the percentage of hemoglobin S is well over 50%, whereas that of hemoglobin A is below 50%. This is in contrast to the sickle cell trait. Although a presumptive diagnosis can be made on this basis, most laboratories usually look for an elevated hemoglobin A_2 level and A_3 low mean corpuscular hemoglobin level as well.

The importance of correctly identifying this variant of sickle cell disease lies not just in the implications for clinical care, but also because of the need to provide correct and appropriate genetic counseling.

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Reply

To the Editors:

We wish to thank Dr. Smith for her comments regarding sickle cell-β-thalassemia. This disorder is about one third as common as sickle cell anemia (homozygosity for Hb S) and produces significant morbidity in some patients. Sickle cell-β+-thalassemia is easily recognized by use of the laboratory data indicated by Dr. Smith. Sickle cell-β-thalassemia is more difficult to identify because hemoglobin A is absent. It can be confused with sickle cell anemia because the hemoglobin electrophoresis for these two disorders is similar. Family and/or hemoglobin biosynthetic studies may be required for differentiation.

Dr. Smith's letter reminds us to emphasize the importance of the level of expertise in the laboratory. Clinicians should communicate with their laboratories to ensure that less common patterns will be recognized and properly interpreted.

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Article does not show harm from midforceps

To the Editors:

In the article "Long-term effects of labor and delivery on offspring: a matched-pair analysis" (AM J OBSTET GYNECOL 1984;150:941), Dr. Friedman and his associates seem to lose sight of what they have proved. In a carefully done analysis, they seem, at least to me, to have confirmed what everyone has known for years, i.e., it is a more hazardous situation for the fetus with more dire long-term effects if the labor is abnormal than if it is normal. That is really all that the article shows. If Dr. Friedman (whom I have greatly admired in the past) feels he is demonstrating the hazards of midforceps deliveries, I fail to see how. I am not sure I can comprehend the type of study that would isolate the hazards of midforceps deliveries (as compared to what?). If the midforceps had not been used, would the patient have delivered "normally" as did the matched pair? Would the patient have been delivered by cesarean section with its attendant risks to both the mother and the infant and in subsequent pregnancies? Would the infant have been damaged more if the labor had continued several hours more?

I think the statisticians are trying to take over the world! We are so lost in complex statistical analyses (which I certainly do not much understand) that we are leaving reason behind. How can one match for analysis of outcome a delivery requiring operative intervention with one that does not? Can we reasonably condemn an emergency tool that has been used with relative safety for more than two generations on the basis of this type of study?

It reminds me a lot of computer analysis. The fact that the computer can manipulate information does not make the information meaningful. Garbage in, garbage out. Does the fact that distinguished and learned men report the results make them any more meaningful?

Perhaps I misunderstand the information or am misinterpreting it, but I fail to see that Dr. Friedman in any way proves his point regarding the safety of midforceps deliveries.

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Reply

To the Editors:

It is nice to learn from Dr. Smith that we "have confirmed what everyone has known for years" about the association between abnormal labor and bad long-term outcome, especially since the issue seems to continue unresolved in some people's minds, all current and prior objective evidence notwithstanding. But that is not the primary thrust of his letter. He questions how we purport to have shown that midforceps procedures cause harm in our matched-pair analysis when we could not have known the impact of not having undertaken the midforceps delivery. Dr. Smith properly rails

against an analysis comparing "the outcome of a delivery requiring operative intervention with one which does not," but we were careful to avoid that pitfall by our method of case selection. All patients included in the analysis were subjected to essentially elective midforceps delivery. Similarly to their matched counterparts who delivered spontaneously, they were free of significant antepartum or intrapartum problems. Except for their abnormal labors, they should not have been expected to do badly. Moreover, the abnormal labor effect was taken into account by ensuring exact matching for the labor pattern (among a number of other factors) in the parallel matched control group. Therefore any adverse long-term effects on the surviving children had to be attributable to the midforceps rather than to the nonexistent indication for the mid-

Another study from this institution clearly showed that the outcome is favorable for infants delivered atraumatically even after a very prolonged second stage. This verified that allowing labor to proceed into the second stage is quite acceptable, even if descent is occurring at an abnormally slow rate (provided appropriate fetal monitoring is done and cephalopelvic disproportion has been ruled out). Thus a useful and apparently safe alternative to midforceps (often done heretofore for no better reason than that second-stage duration had exceeded 2 hours) is expectancy and careful surveillance.

The argument then is not whether midforceps delivery prevents damage that might occur from allowing the labor to continue, but whether it inflicts damage that would not have occurred had the patient been managed more expectantly. As to Dr. Smith's question about condemning an "emergency tool," we assiduously avoided the question of balance of risks because we recognized the great difficulty of simultaneously weighing the risk of a condition (such as fetal distress) against the counterbalancing risk of a procedure (such as midforceps delivery) done to avoid the first risk. Our objective was to try to determine the magnitude of the risk of operative vaginal delivery when done in cases in which the risk of the indication for the procedure was not at issue, thereby averting its confounding effects. The hazard has now been quantified; those who wish to invoke that risk by using the tool for an emergency are well advised to ensure (and document) that the risk of the emergency exceeds that of the procedure (or available alternatives).

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REFERENCE

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In defense of midforceps

To the Editors:

As a full-time academic obstetrician for more than a generation, I take exception to the paper by Friedman et al. (Long-term effects of labor and delivery on offspring: a matched-pair analysis. Am J Obstet Gynecol 1984;150:941), particularly their discussion on the role of midforceps in modern-day obstetrics. I believe that we should continue to teach house staffs how to perform midforceps delivery. I have reviewed two cases of malpractice suits for cerebral palsy in which, with the cervix fully dilated, for fetal distress the obstetrician chose to undertake a cesarean section rather than attempt an immediate midforceps delivery. In both cases their unsuccessful defense was that they had not been taught how to perform a midforceps delivery because it was no longer a modern obstetric technique.

Like Richardson et al., I have also been suspicious of the National Collaborative Perinatal Project's forceps data. As a resident and faculty person in a university hospital that participated in this study, I recall that we rarely performed midforceps deliveries and when we did, it was under unfavorable conditions. Contrary to the Friedman study, Nilsen, in a 1984 study of boys delivered by Kjelland forceps and examined at 18 years of age, found a significant elevated mean intelligence score in those delivered by forceps.

One of the disadvantages of Dr. Friedman's emphasis on the harm of the midforceps delivery is its encouragement of lack of objectivity. For instance, there are hospitals in Omaha where a high percentage of laboring women receive epidural anesthesia. Yet according to their labor room log book, the obstetricians manage to deliver their babies with only low forceps. In my experience, any service with a high percentage of use of effective epidural anesthesia, by the standard definitions, must frequently use midforceps! I believe such midforceps operations are justifiable and should be acknowledged.

Robert C. Goodlin, M.D.

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Reply

To the Editors:

There is an inherent fallacy in Dr. Goodlin's logic. He is advocating continuing a practice for teaching purposes which he feels is useful because it can sometimes prevent harm and serve as a substitute for cesarean section. When the fetal risks are great and the need

urgent, some of the harm accruing from the prozedure could perhaps be justified, although even that is arguable. The skill cannot be learned, however, verhout undertaking the procedure in cases in which it may not be so clearly indicated, if indicated at all. Can the harm to the fetus be justified by a counterbalancing renefit to the individual under such circumstances? Cearly not. Our obstetric practices have to be governed by what is best for the specific patient, weighing the rist and benefits for her and her fetus. In the overview we cannot accept exposing many patients to a risk in order to teach a procedure that might (or might not) be fit a few.

As to midforceps use for cases with epidural anesthesia, we have long recommended allowing second-stage labor to continue until atraumatic delivery—ould be effected; such patients need strong nursing support and encouragement for effective expulsive effor. Alternatively the anesthesia could be permitted to soate. The good results from such a policy have recently seen verified by a British study.

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The role of midforceps in current obstetric practice

To the Editors:

In the final paragraph of their recent article (Lengterm effects of labor and delivery on offspring: a matched-pair analysis. Am J OBSTET GYNECOL I 84; 50:941), Dr. Friedman et al. indicate that they have given the final proof to their contention that mi-Eorceps procedures should no longer be done exce in unusual circumstances. While most clinicians rave more or less agreed, we still need a clear demonstration that the alternative management produces bette-results. The alternative in most instances is, of course, cesarean section and not spontaneous vaginal delizery. In fact, the normal spontaneous vaginal delivery outcome in his control group in the current paper sug sts there still may be a significant undefined differ nce between this group and those requiring operative intervention.

Do Dr. Friedman and his associates have any ata that address this issue? Certainly a comparable ontrolled study proving a better intellectual long-term-result in the child delivered by cesarean section instand of midforceps delivery would seem to be the definative answer to the question.

The importance of this further study cannot be car-

emphasized, since there are obvious medicolegal as well as clinical implications.

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Reply

To the Editors:

Dr. Pearson has overstated our position, perhaps to provide a straw man to do battle with. We do not contend we have provided "the final proof." Indeed we state in the introduction that there are insurmountable ethical barriers to obtaining such proof. It would nevertheless have been valuable to offer some additional confirmatory substantiation in the form of matched cesarean section cases. However, by confining ourselves in the current study to essentially uncomplicated cases (except for the labor disorder and delivery mode under study), we were left with insufficient numbers delivered by cesarean section to match. It is hoped that the more complex multivariate analyses now being applied to the large numbers of relevant labor-delivery variables will help clarify this issue.

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Idiopathic nonimmune hydrops: A common entity

To the Editors:

The article by Holzgreve W et al. (Investigation of nonimmune hydrops fetalis. Am J OBSTET GYNECOL 1984;150:805-12) made some excellent points but also left some impressions that I must take issue with.

I do not think that their reported rate (84%) for successfully establishing a cause for nonimmune hydrops fetalis is accurate. First, they excluded all cases in which the fetus was not alive when studied or in which complete follow-up was not available. They do not tell us what the number of excluded cases is. If this number were to be considered, it could significantly alter the rate for successful determination of the etiology of hydrops fetalis. Second, Holzgreve et al. have also not sufficiently proved a cause-and-effect relationship between what they have listed as an etiology in some cases and the occurrence of hydrops fetalis. Some of the cases that raise that question include Cases 4, 10, 11, 27-32, and 49.

In an excellent review Machin² summarized several moderate to large series of cases of hydrops fetalis. The investigation scheme laid out in that summary is very similar to the one presented by Holzgreve et al. yet the idiopathic rate is 50 of 117 cases, or 43%. There are other series that report idiopathic rates in the 40% to 50% range, and I feel that this more accurately reflects

the difficulty in establishing a true etiology for non-immune hydrops fetalis.

Overall, this is an excellent article, which makes two important points: First, the suggestion, also made by others, ¹⁻⁴ that both the relative and absolute incidence of nonimmune hydrops is increasing. Second, the conclusion that a thorough investigation, as presented, is essential if an etiology for any case of hydrops fetalis is to be found.

Nonimmune hydrops is an intriguing problem that practitioners will have to face with increasing frequency. Each case should be investigated for an etiology in an orderly, thorough fashion. At present, it is more realistic to expect to find an etiology in approximately 50% to 60% of the cases and not 84% of cases as Holzgreve et al. suggest.

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Reply

To the Editors:

Dr. Buttino is concerned about the 84% rate of establishing an etiology for nonimmune hydrops fetalis reported in our article. We agree that an absolute causeand-effect relationship cannot be proved for many such cases but to assume no relationship is to require the fetus to have two rare findings, an unlikely situation. The cases of fetal death were excluded because we feel that the dying fetus may lose capillary integrity and become edematous as part of the death process. We have now extended our series to 103 cases of nonimmune hydrops (soon to appear in Seminars in Perinatology), with a causal relationship suggested for 87 fetuses (84.5%). I would maintain that if the thorough investigation we and others have urged is carried out, it is then quite realistic to find a probable etiology in 80% of the cases of nonimmune hydrops.

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Proper documentation of hemoglobinopathies

To the Editors

We will accept that the patient in this very important report (Pastorek JG, Seiler B. Maternal death associated with sickle cell trait. Am J OBSTET GYNECOL 1985; 151:295-7) has hemoglobin AS (sickle cell trait) because the authors so state.

Unfortunately, they do not detail how this diagnosis was made. Too often a simple positive sickle cell preparation is assumed to be hemoglobin AS. This implies that the pregnant patient is symptom-free at sea level and always has less than 50% hemoglobin S in erythrocytes, the remainder being hemoglobin A. Of more than 300 known abnormal hemoglobins, at least three (C, D, O_{Arab}) aggravate sickling and should be documented in a case report. Hereditary persistence of hemoglobin F in a significant concentration protects against sickling and may produce a mild, if any, adverse clinical picture in certain populations.

We assume that a hemoglobin electrophoresis was performed. If so, percentages of hemoglobins A, S, and A_2 should be listed. If not, the diagnosis suffers from imprecise identification of the hemoglobinopathy.

Clinical features of hemoglobin SA are usually worse than hemoglobin AS. Heterozygous β -thalassemia could have contributed to the course, although A_2 levels, mean corpuscular volume, and red cell distribution width are not reported.

It is more logical to assume (as Pastorek and Seiler correctly relate) that this is a maternal death in a rather ill patient who incidentally has hemoglobin AS. With the exception of urinary tract infection, interaction of sickle trait and pregnancy is constantly benign. Again, this is another reason for full documentation of hematologic status.

It is interesting to note that Pastorek and Seiler do not describe sickling in the placenta. This is not a minor oversight of the paper. Details of hematologic findings should be documented. Further observation and investigation of this critical topic should be conducted, as the authors correctly write. However, unfortunately this would be based on incomplete case reports such as the present one.

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REFERENCE

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Reply

To the Editors:

We appreciate the comments by Almeida and Kitay regarding specific diagnosis of sickle trait in our patient.

It is certainly important to exclude other hematologic abnormalities in cases such as this, since the whole point of this presentation was to connect sickle trait itself with the complications described. Certainly, should the patient under discussion suffer any complicating conditions (hemoglobin C, hemoglobin O, persistence of hemoglobin F, etc.), the story would be quite confusing and no clear statement could be made regarding sickle cell trait itself.

To answer the question, the patient presented in this unfortunate case was determined to have sickle cell trait on the basis of a double electrophoresis: a cellulose acetate/alkaline buffer electrophoresis followed by a citrate/acid buffer electrophoresis. Exact quantitation of percent of hemoglobin A, S, and A_2 was not routinely done, since the patient was believed to have sickle cell trait. Red blood cell indices are available: mean corpuscular volume, 93.6 μ^3 ; mean corpuscular hemoglobin concentration, 34.0%; mean corpuscular hemoglobin, 31.8 $\mu\mu g$.

Considering the above laboratory findings, we are confident that this patient did indeed have only sickle cell trait uncomplicated by thalassemia or less common hemoglobinopathies. However, Almeida and Kitay make an excellent point in that the diagnosis of sickle cell trait cannot be made on the basis of a positive "sickle cell preparation" alone but rests on hemoglobin electrophoretic patterns.

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Tocolysis of eclampsia-associated uterine hypertonus

To the Editors:

Barrett (Fetal resuscitation with terbutaline during eclampsia-induced uterine hypertonus. Am J Obstet Gynecol 1984;150:895) reported on the use of a β-mimetic in treating eclampsia-induced hypertonus with associated prolonged fetal bradycardia. Magnesium sulfate is accepted therapy for treating preeclampsia/eclampsia. It has been shown to be an effective tocolytic in response to uterine hypertonus.¹

In the case report there is no mention of the docage or temporal relationship of magnesium sulfate administration to the convulsion. Rather than the suggestion that diazepam and terbutaline be used in response to an eclamptic seizure, certainly an intravenous bolus of magnesium sulfate would be a better choice as a means of treating both the seizure and the associated uterine hypertonus.

David N. Kells, M.D.

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REFERENCE

 Reece E, Chervinak F, Romero R., et al. Magnesium sulfate in the management of acute intrapartum fetal distress. Am J OBSTET GYNECOL 1984;148:104.

Reply

To the Editors:

Dr. Kells' interest in the case report on fetal resuscitation with terbutaline during eclampsia-induced uterine hypertonus is very much appreciated. In response to his questions, the patient had been on magnesium sulfate for 6 hours prior to the eclamptic seizure. The magnesium sulfate had been given with an initial dose of 4 gm administered intravenously over 15 minutes followed by a continuous intravenous infusion of 1 gm of magnesium sulfate per hour. At the time this patient had the eclamptic seizure a serum magnesium level was pending.

The reason for not giving 2 to 4 gm of magnesium sulfate intravenously was twofold. First, there was the concern that should the patient's magnesium level already be elevated, respiratory depression might occur from giving more magnesium sulfate rapidly. The second is that it is my opinion that the use of magnesium sulfate as a tocolytic agent has yet to be adequately proved whereas the use of β -mimetic agents has been well documented.

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More on malignant hyperthermia during delivery

To the Editors:

The report of a case of malignant hyperthermia in pregnancy by Cupryn et al. (Cupryn JP, Kennedy A, Byrick R.J. Malignant hyperthermia in pregnancy. Am J OBSTET GYNECOL 1984;150:327) raises several questions. The first pertains to the incidence and severity of malignant hyperthermia during delivery. The issue of malignant hyperthermia reactions during pregnancy was raised in the British literature in 1972. We have estimated conservatively that between 1972 and 1982 the number of deliveries with general anesthesia in the United States, Canada, and Great Britain (three countries actively studying malignant hyperthermia) was 2,931,300. (Our estimate is based on census data of births, published rates of cesarean section, and an estimated 50% rate of general anesthesia for cesarean section.) Agents that trigger malignant hyperthermia (succinylcholine and potent inhalational agents) are used frequently. If the incidence of malignant hyperthermia among adults is 1:52,000 administrations of anesthesia as Cupryn et al. state, then there should have been 56 reactions associated with delivery during this period. Since 20%2 to 50% (Cupryn et al.) of malignant hyperthermia reactions are fatal, 11 to 28 pregnant patients should have died of malignant hyperthermia. If the stress of labor in unanesthetized patients and the use of local amide anesthetics also can trigger malignant hyperthermia, then the incidence of malignant hyperthermia reactions and mortality should be several times higher than the number calculated above. We find it curious then that only four cases of malignant hyperthermia (Cupryn et al.) and no fatalities³ during delivery have been reported. In 1972 Crawford¹ noted that pregnancy may have a protective effect against malignant hyperthermia. The above statistics support that possibility.

The second question involves the prophylactic use of dantrolene in malignant hyperthermia—susceptible patients at the time of delivery. Dantrolene crosses the placenta with unknown fetal and neonatal effects. Immaturity of the reconatal neuromuscular system may increase the sensitivity to the muscle relaxant effects of dantrolene and thus may be deleterious. We therefore question the safety and necessity of empiric dantrolene prophylaxis.

We have also cared for malignant hyperthermia—susceptible patients during delivery. We use agents not associated with malignant hyperthermia for general or regional anesthesia. Dantrolene is not given prophylactically, but the patient is carefully monitored and preparation made to administer dantrolene at the first sign of malignant hyperthermia. Also, we determine umbilical artery, umbilical venous, and maternal arterial blood gases and creatine phosphokinase levels at the time of delivery to help diagnose a malignant hyperthermia reaction in either the mother or the newborn. With this protocol no reactions have occurred during three deliveries with two malignant hyperthermia—susceptible patients.

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Kenneth R. Kellner, M.D., Ph.D.

Department of Obstetrics and Gynecology University of Florida College of Medicine Gainesville, Florida 32610-0294

REFERENCES

- Crawford JS. Hyperpyrexia during pregnancy. Lancet 1972;1:1244.
- 2. Britt BA. Dantrolene. Can Anaesth Soc J 1984;31:61-75.
- Tettambel M. Malignant hyperthermia in an obstetric patient. JAOA 1980;79:773-775.
- Morison DH. Placental transfer of dantrolene [Letter]. Anesthesiology 1983;59:265.

Reply

To the Editors:

We agree with Kaplan and Kellner that the rarity of a malignant hyperthermia reaction in parturient women may suggest a protective gestational effect. However, the potential severity of such a reaction has been reported in a relative of Wadhwa's patient; in this case both mother and fetus died.1

The preoperative use of dantrolene is controversial, and as we stated, "safety to the fetus has not been established." The clinician weighs the potential advantages² against potential deleterious effects of the drug. Further research will clarify this risk-benefit decision and the optimal protocol to be used.

Meanwhile, we appreciate and value the experience of Kaplan and Kellner and stress that malignant hyperthermia—susceptible patients should not be advised to avoid pregnancy.

J. P. Cupryn, M.D. A. Kennedy, M.D. R. J. Byrick, M.D.

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REFERENCES

- Wadhwa RK. Obstetric anesthesia for a patient with malignant hyperthermia susceptibility. Anesthesiology 1977; 46:63.
- Gronert GA. Malignant hyperthermia. Anesthesiology 1980;53:395.

Premature rupture of membranes at term

To the Editors:

In their recent article, "Management of spontaneous rupture of the membranes in the absence of labor in primigravid women at term" (AM J OBSTET GYNECOL 1984;150:947-51) Conway et al. compared spontaneously occuring labor to induced and augmented labor and demonstrated an increased rate of abdominal delivery in the group receiving oxytocin. Based on their results, they advocate awaiting spontaneous labor for 24 hours prior to considering induction of labor in these patients.

There are several problems with this study and its conclusions. The initial allocation of patients to groups was similar to Kappy et al.1 and Duff et al.2 in that patients with induced labors were compared to spontaneously laboring patients. This allocation of patients is improper to test their hypothesis. It is quite possible (if not probable) that the patients who promptly commence labor following rupture of membranes are from a population very different from the population that does not labor. The proper selection of patients for testing of whether early induction of labor increases the risk of abdominal delivery is one that randomizes into two groups only those patients who fail to commence labor by a specified interval (such as 6 hours). By necessity this would reduce the number of patients who would qualify for the study but would strengthen the logic and conclusions of the trial.

Once Conway et al. assigned the patients to spontaneously laboring and nonlaboring groups, they altered their results (and therefore their conclusions) by an analytic maneuver that seems questionable. From the

spontaneously laboring group they extracted those that required augmentation of labor and analyzed them with the induced group. They effectively extracted most of their likely dystocia patients (laboring patients requiring augmentation) from their control group before making the comparison to patients with induced labor. This regrouping of patients departs from their design to compare spontaneous to induced labor.

Conway et al. conclude by recommending a 24-hour delay before considering induction of labor in these patients. This conclusion is not investigated by their study design nor is it directed from their results.

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REFERENCES

- Kappy KA, Cetrulo CL, Knuppel RA, et al. Premature rupture of the membranes at term. J Reprod Med 1982;27:29-33.
- 2. Duff D, Huff RW, Gibbs RS. Management of premature rupture of membranes and unfavorable cervix in term pregnancy. Obstet Gynecol 1984;63:697-702.

Reply

To the Editors:

Thank you for the opportunity to reply to Γ r. Spinnato's letter concerning our spontaneous rupture of

membranes trial (AM J OBSTET GYNECOL 1984;150: 947-51). It is true that our allocation method was not random and also that to do so would dramatically reduce the number of patients we were able to study. We agree that it is not possible to say that the risk of operative delivery is truly decreased in patients who are not electively induced. However, although the design of our study was not random, it was a nonselective method (unless a circadian rhythm operates). The subsequent division of noninduced patients into those augmented and those not augmented does not detract from the fact that 94% of those who went into spontaneous labour were delivered vaginally compared to 73% of the induced group (p = <0.01).

More importantly our study did show that the risk of infection in our study group was low. Therefore the *indication* for intervention evaporates. Clinicians who advocate intervention should now demonstrate the advantages of intervention (perhaps by a randomized trial).

Walter J. Prendiville David I. Conway

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Books received

- Abortion. Understanding Differences. Edited by Sidney Callahan and Daniel Callahan. 338 pages. New York, 1984, Plenum Publishing Corporation. \$35.00.
- Actualidades en Medicina Perinatal. Pedro J. Faneite Antique. 222 pages, illustrated. Puerto Cabello, Venezuela, 1984, B. Alder. No price listed (soft cover).
- Ambulatory Gynecology. David H. Nichols and John R. Evrard. 550 pages, illustrated. Philadelphia, 1984, Harper and Row, Inc. \$35.00.
- Antibiotic Prophylaxis in Surgery. A Comprehensive Review. John E. Conte, Jr., Leonard S. Jacob, and Hiram C. Polk, Jr. 196 pages, illustrated. Philadelphia, 1984, J. B. Lippincott Company. \$22.50.
- The Biochemical Effects of Drugs in Pregnancy. Volume II. A. Onnis and P. Grella. 493 pages, illustrated. New York, 1985, John Wiley & Sons, Inc. \$88.95.
- Birth Over Thirty. Sheila Kitzinger. 182 pages, illustrated. New York, 1985, Penguin Books. \$5.95 (soft cover).
- Care of the Postmenopausal Patient. Edited by Frederick J. Hofmeister. 194 pages, illustrated. Philadelphia, 1985, George F. Stickley Company. \$15.00 (soft cover).
- Clinics in Obstetrics and Gynaecology. Volume 11, Number 3. John R. Newton, guest editor. 270 pages, illustrated. Philadelphia, 1984, W. B. Saunders Company. No price listed.
- Contemporary Obstetrics. Edited by Geoffrey Chamberlain. 293 pages, illustrated. Stoneham, Massachusetts, 1984, Butterworth Publishers. \$39.95 (soft cover).

- Contemporary Gynaecology. Edited by Geoffrey Chamberlain. 314 pages, illustrated. Stoneham, Massachusetts, 1984, Butterworth Publishers. \$35.00 (soft cover).
- Immunology in Obstetrics and Gynecology. James R. Scott and Neal S. Rote. 286 pages, illustrated. Norwalk, Connecticut, 1985, Appleton-Century-Crofts. No price listed.
- Left Brain, Right Brain. Revised Edition. Sally P. Springer and Georg Deutsch. 320 pages, illustrated. New York, 1985, W. H. Freeman and Company. \$11.95 (soft cover).
- The Medical Management of Menopause and Premenopause. Winnifred Berg Cutler and Celso-Ramon Garcia. 275 pages, illustrated. Philadelphia, 1984, J. B. Lippincott Company. \$27.50.
- Physiologic Foundations of Perinatal Care. Edited by Leo Stern, Marietta Xanthous, and Bent Friis-Hansen. 386 pages, illustrated. New York, 1985, Praeger. \$45.00.
- Practical Colposcopy. Rene Cartier. 320 pages, illustrated. Paris, 1984, Laboratoire Cartier. FF 980.
- **Proper Doctoring.** David Mendel. 177 pages. New York, 1984, Springer-Verlag. \$9.50 (soft cover).
- Viral Diseases of the Fetus and Newborn. Second Edition. James Barry Hanshaw, John Alastair Dudgeon, and William C. Marshall. 336 pages, illustrated. Philadelphia, 1985, W. B. Saunders Company. \$39.95.

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- Intensive Board Review Course in Reproductive Endocrinology, September 13-16, 1985, Massachusetts General Hospital, Boston, Massachusetts. Contact: Dr. William F. Crowley, Jr., Vincent Research Laboratories, Massachusetts General Hospital, Boston, MA 02114.
- Three Postgraduate Gynecology Courses: Advanced Gynecologic Oncology, September 7-9, 1985; Advances in Gynecology, September 10-12, 1985; Reproductive Endocrinology, September 13-16, 1985; Massachusetts General Hospital, Boston, Massachusetts. Contact: Harvard Medical School, Department of Continuing Education, 25 Shattuck St., Boston, MA 02115.
- London and Paris Fall Ultrasound—Obstetrics and Gynecology, London, England, September 20-25, 1985; Paris, France, September 25-29, 1985. For further information contact: Secretary, Fall Ultrasound Symposia, Medical Seminars International, West Park Medical Office Building, 22135 Roscoe Blvd., #104, Canoga Park, CA 91304. Tel.: (818) 340-0580, ext. 280.
- A Clinical and Histopathologic Overview of Obstetrics and Gynecology, September 24-28 1985,

- New York Hilton, New York, New York. For further information contact: Department of Obstetrics and Gynecology, Saint Barnabas Medical Center, Livingston, NJ 07039.
- Specialty Review in Obstetrics and Gynecology, October 28—November 2, 1985, Chicago, Illinois. For further information contact: Dick Nelson, Course Manager, The Cook County Graduate School of Medicine, 707 South Wood St., Chicago, IL 60612. Tel.: (312) 633-2600.
- Perinatal Malpractice, September 12-13, 1985, Northwestern University Medical School Alumni Center for Continuing Education, 301 E. Chicago Ave., Chicago, IL 60611. For further information contact: Paula Puntenny, Director, Alumni Center for Continuing Education, Northwestern University Medical School, 301 E. Chicago Ave., Chicago, IL 60611. Tel.: (312) 908-8533.
- Laser Update—Gynecology, Plastic Surgery, Dermatology, September 12-13, 1985. University of California, Irvine. For further information contact: Ann Siemans, 391 Steinhaus Hall, University of California, Irvine, CA 92717. Tel.: (714) 856-7033; 856-6291.

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WARNINGS

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penicillinase producing).

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Precautions: Total daily dose should be reduced in patients with reduced urinary output due to renal insufficiency because high and prolonged serum antibiotic concentrations can occur from usual doses. Prescribe with caution in patients with a history of gastrointestinal disease, particularly colitis. Prolonged use may result in overgrowth of nonsusceptible organisms; repeated evaluation of the patients condition is essential. If superinfection occurs, take appropriate measures. Increased nephrotoxicity has been reported following concomitant administration of cephalosporins and amino

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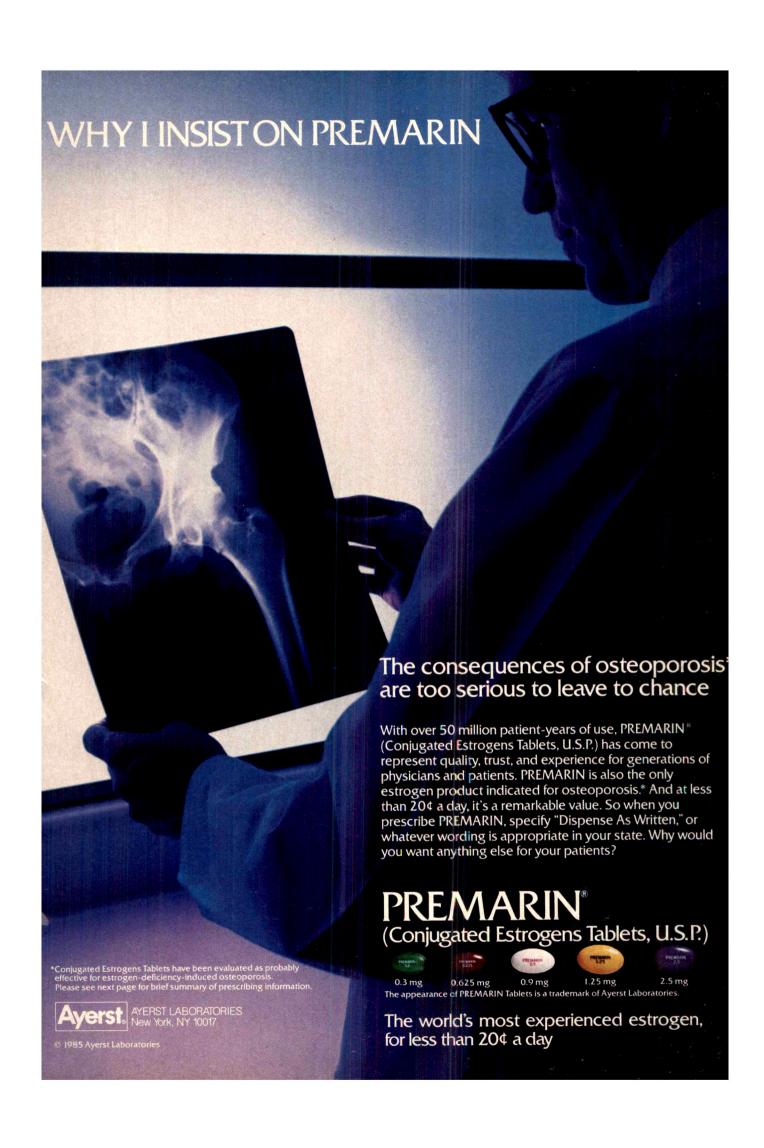
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PREMARIN® Brand of Conjugated Estrogens Tablets, U.S.P.

1. ESTROGENS HAVE BEEN REPORTED TO INCREASE THE RISK OF ENDOMETRIAL CARCINOMA.

Three independent case control studies have reported an increased risk of endometrial cancer in postmenopausal women exposed to exogenous estrogens for more than one year. This risk was independent of the other known risk factors for endometrial cancer. This risk was independent of the other known risk factors for endometrial cancer have increased sharply since 1969 in eight different areas of the United States with population-based cancer reporting systems, an increase which may be related to the rapidly expanding use of estrogens during the last decade. The three case control studies reported that the risk of endometrial cancer in estrogen users was about 4.5 to 13.9 times greater than in nonusers. The risk appears to depend on both duration of treatment and on estrogen dose. In view of these findings, when estrogens are used for the treatment of menopausal symptoms, the lowest dose that will control symptoms should be utilized and medication should be discontinued as soon as possible. When prolonged treatment is medically indicated, the patient should be reassessed on at least a semiannual basis to determine the need for continued therapy. Although the evidence must be considered preliminary, one study suggests that cyclic administration of low doses of estrogen may carry less risk than continuous administration, it therefore appears prudent to utilize such a regimen. Close clinical surveillance of all women taking estrogens is important. In all cases of undiagnosed persistent or recurring abnormal vaginal bleeding, adequate diagnostic measures should be undertaken to rule out malignancy. There is no evidence at present that "natural" estrogens are more or less hazardous than "synthetic" estrogens at equiestrogens and progestogens, during early pregnancy may seriously damage the offspring. It has been shown that temales exposed in utero to diethylstilibestrol. a non-steroid

DESCRIPTION: PREMARIN (Conjugated Estrogens Tablets, U.S.P) for oral administration contains a mixture of estrogens, obtained exclusively from natural sources, blended to represent the average composition of material derived from pregnant mares urine. It contains estrone, equilin, and 17 α -dihydroequilin, together with smaller amounts of 17 α -estradiol, equilenin, and 17 α -dihydroequilenin as salts of their sulfate esters.

INDICATIONS: Based on a review of PREMARIN Tablets by the National Academy of Sciences—National Research Council and/or other information, FDA has classified the ications for use as follows:

1. Moderate to severe vasamotor symptoms associated with the menopause (There is no evidence that estrogens are effective for nervous symptoms or depression without associated vasamotor symptoms, and they should not be used to treat such conditions.)

Atrophic vaginitis. Kraurosis vulvae

3. Kraurosis vulvae.
4. Female hypogonadism.
5. Female castration.
6. Primary ovarian failure.
7. Breast cancer (for palliation only) in appropriately selected women and men with metastatic disease.
8. Prostatic carcinoma—palliative therapy of advanced disease.
9. Postpartum breast engorgement—Although estrogens have been widely used for the prevention of postpartum breast engorgement, controlled studies have demonstrated that the incidence of significant painful engorgement in patients not receiving such hormonal therapy is low and usually responsive to appropriate analgesic or other supportive therapy. Consequently, the benefit to be derived from estrogen therapy for its indication must be carefully weighed against the potential increased risk of puerperal thromboembolism associated with the use of large doses of estrogens. PREMARIN HAS NOT BEEN SHOWN TO BE EFFECTIVE FOR ANY PURPOSE DURING PREGNANCY AND ITS USE MAY CAUSE SEVERE HARM TO THE FETUS (SEE BOXED WARNING).

BOXED WARNING).
"Probably" effective: For estrogen deficiency-induced osteoporosis, and only when used in conjunction with other important therapeutic measures such as ciet, calcium, physiotherapy, and good general health-promoting measures. Final classification of this indication requires further investigation.

CONTRAINDICATIONS: Estrogens should not be used in women (or men) with any of the following conditions: 1. Known or suspected cancer of the breast except in appropriately selected patients being treated for metastatic disease. 2. Known or suspected estrogen-dependent neoplasia. 3. Known or suspected pregnancy (See Boxed Warning). 4. Undiagnosed abnormal genital bleeding, 5. Active thrombophlebitis or thromboembolic disorders. 6. A past history of thrombophlebitis, thrombosis, or thromboembolic disorders associated with previous estrogen use (except when used in treatment of breast or prostatic malignancy).

nancy). WARNINGS: Long term continuous administration of natural and synthetic estrogens in certain animal species increases the frequency of carcinomas of the breast, cervix, vagina, and liver. There are now reports that estrogens increase the risk of carcinoma of the endometrium in humans. (See Boxed Warning.) At the present time there is no satisfactory evidence that estrogens given to postmenopausal women increase the risk of cancer of the breast, alterdough a recent study has raised this possibility. There is a need for caution in prescribing estrogens for women with a strong family history of breast cancer or who have breast nodules, florcystic disease, or abnormal mammograms. A recent study has reported a 2- to 3-fold increase in the risk of surgically confirmed gallbladder disease in women receiving postmenopausal estrogens.

Adverse effects of oral contraceptives may be expected at the larger doses of estroge used to treat prostatic or breast cancer or postpartum breast engorgement; it has bee shown that there is an increased risk of thrombosis in men receiving estrogens for prostatic cancer and women for postpartum breast engorgement. Users of oral contraceptives have an increased risk of diseases, such as thrombophiebitis, pulmonary embolism, stroke, an myocardial infarction. Cases of retinal thrombosis, mesenteric thrombosis, and optic neur tis have been reported in oral contraceptive users. An increased risk of postsurgery thromboembolic complications has also been reported in users of oral contraceptives. If feasible estrogen should be discontinued at least 4 weeks before surgery of the type associated wit an increased risk of thromboembolism, or during periods of prolonged immobilization. Es trogens should not be used in persons with active thrombophiebitis, thromboembolic disor ders, or in persons with a history of such disorders in association with estrogen use. The should be used with caution in patients with cerebral vascular or coronary artery disease. Large doses (5 mg conjugated estrogens per day), comparable to those used to treat care of the prostate and breast, have been shown to increase the risk of nonfatal myocardic infarction, pulmonary embolism and thrombophlebitis. When doses of this size are used, an of the thromboembolic and thrombotic adverse effects should be considered a clear risk. Benign hepatic adenomas should be considered in estrogen users having abdomina pain and tenderness, abdominal mass, or hypovolemic shock. Hepatocellular carcinomas been reported in women taking estrogens in the menopause and blood pressure may occur with use of estrogens in the menopause and blood pressure holded be monitored with estrogen user. A worsening of glucose tolerance has been observed in patients on estrogen certainly observed. Estrogens may lead to severe hypercalcemia in patient with breast cancer and bone metasta

served in patients on estrogen-containing oral contraceptives. For this reason, diabetic patients should be carefully observed. Estrogens may lead to severe hypercalcemia in patient with breast cancer and bone metastases.

PRECAUTIONS: Physical examination and a complete medical and family history shoult be taken prior to the initiation of any estrogen therapy with special reference to blood pressure, breasts, abdomen, and pelvic organs, and should include a Papanicolau smear. As i general rule, estrogen should not be prescribed for longer than one year without anothe physical examination being performed. Conditions influenced by fluid retention such a asthma, epilepsy, migraine, and cardiac or renal dysfunction, require careful observation. Certain patients may develop manifestations of excessive estrogenic stimulation, such as asthma, epilepsy, migraine, and cardiac or renal dysfunction, require careful observation. Certain patients may develop manifestations of excessive estrogenic stimulation, such as abnormal or excessive uterine bleeding, mastodynia, etc. Prolonged administration of unop posed estrogen therapy has been reported to increase the risk of endometrial hyperplasia in some patients. Oral contraceptives appear to be associated with an increased incidence of merital depression. Patients with a history of depression should be carefully observed. Pre existing uterine leiomyomata may increase in size during estrogen use. The pathologis should be advised of estrogen therapy when relevant specimens are submitted. If jaundia develops in any patient receiving estrogen, the medication should be discontinued while the cause is investigated. Estrogens should be used with care in patients with impaired live function, renal insufficiency, metabolic bone diseases associated with hypercalcemia, or ir young patients in whom bone growth is not complete.

The following changes may be expected with larger doses of estrogen:

a lncreased sulfobromophthalien retention.

b. Increased sulfobromophthalien retention.

b.

ACUTE OVERDUSAGE: May cause nausea, and withdrawal bleeding may occur in females.

DOSAGE AND ADMINISTRATION: 1. Given cyclically for short-term use only: For treatment of moderate to severe vasomotor symptoms, atrophic vaginitis, or kraurosis vulvae associated with the menopause (0.3 to 1.25 mg or more daily).

The lowest dose that will control symptoms should be chosen and medication should be discontinued as promptly as possible.

Administration should be cyclic (e.g., three weeks on and one week off).

Attempts to discontinue or taper medication should be made at three to six month intervals.

2. Given cyclically: Female hypogonadism. Female castration. Primary ovarian failure

2. Given cyclically. Female hypogonadism. Female castration. Primary ovarian failure. Osteoporosis.

Female hypogonadism—2.5 to 7.5 mg daily, in divided doses for 20 days, followed by a rest period of 10 days' duration. If bleeding does not occur by the end of this period, the same dosage schedule is repeated. The number of courses of estrogen therapy necessary to produce bleeding may vary depending on the responsiveness of the endometrium. If bleeding occurs before the end of the 10 day period, begin a 20 day estrogen-progestin cyclic regimen with PREMARIN (Conjugated Estrogens Tablets, U.S.P.), 2.5 to 7.5 mg daily, cyclic regimen doses, for 20 days. During the last five days of estrogen therapy, give an oral progestin. If bleeding occurs before this regimen is concluded, therapy is discontinued and may be resumed on the fifth day of bleeding.

Female castration and primary ovarian failure—1.25 mg daily, cyclically. Adjust upward or downward according to response of the patient. For maintenance, adjust dosage to lowest level that will provide effective control.

Osteoporosis (to retard progression)—1.25 mg daily, cyclically.

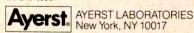
3. Given for a few days: Prevention of postpartum breast engorgement—3.75 mg every four hours for five doses, or 1.25 mg every four hours for five days.

4. Given chronically: Inoperable progressing prostatic cancer—1.25 to 2.5 mg three times daily.

Inoperable progressing breast cancer in appropriately selected men and postmenopaus-al women—10 mg three times daily for a period of at least three months. Patients with an intact uterus should be monitored for signs of endometrial cancer and ap-propriate measures taken to rule out malignancy in the event of persistent or recurring abnor-mal vaginal bleeding.

Propriate measures taken to rule out malignancy in the event of persistent or recurring abnormal vaginal bleeding.

HOW SUPPLIED: PREMARIN (Conjugated Estrogens Tablets, U.S.P.). No. 865—Each purple tablet contains 2.5 mg in bottles of 100 and 1,000. No. 866—Each yellow tablet contains 1.25 mg in bottles of 100 and 1,000. Also in Cycle Pack of 21 and in unit dose package of 100. No. 864—Each white tablet contains 0.9 mg in bottles of 100. Also in Cycle Pack of 21. No. 867—Each marzon tablet contains 0.625 mg in bottles of 100 and 1,000. Also in Cycle Pack of 21 and unit dose package of 100. No. 868—Each green tablet contains 0.3 mg in bottles of 100 and 1,000.





American Journal of Obstetrics and Gynecology

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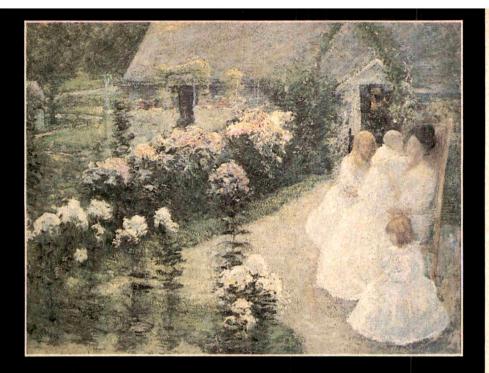
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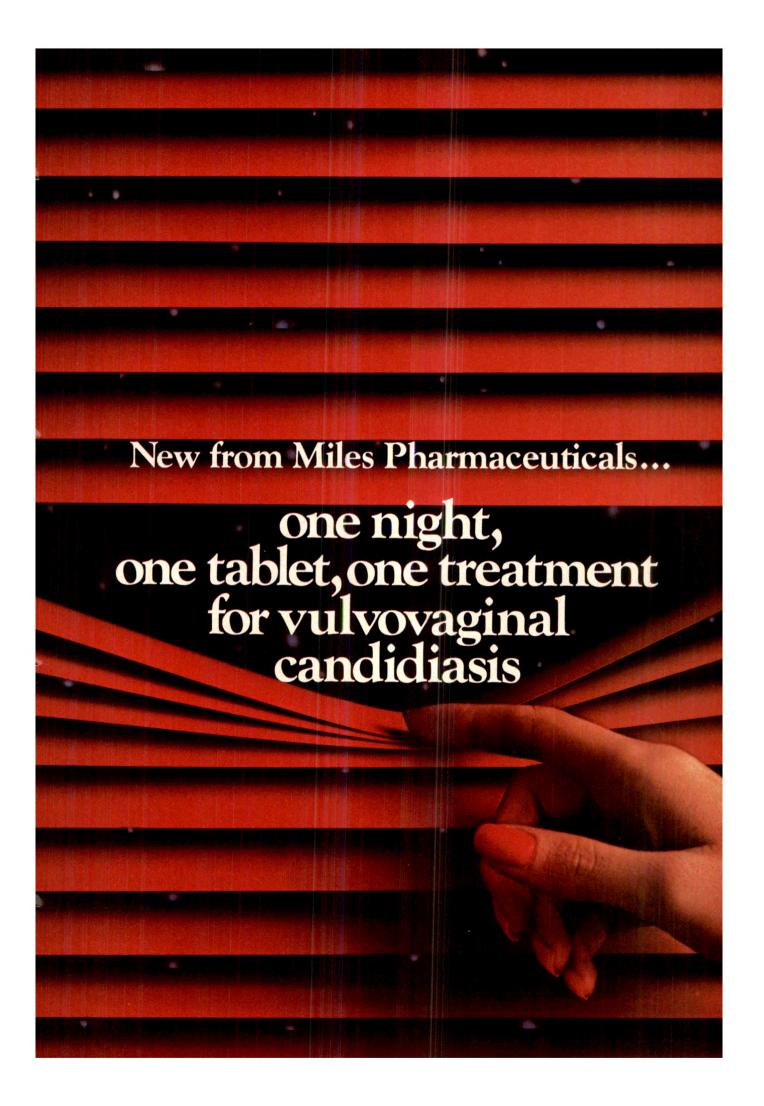
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References:

1. Ritter W, Patzschke K, Krause U, et al: Pharmacokinetic fundamentals of vaginal treatment with clotrimazole, Chemotherapy 28 (Suppl 1):37, 1982. 2. Holl RJ, Newman RL: J Clin Path 25:1089, 1972. 3. Mendling W, Plempel M: Vaginal secretion levels after 6 days, 3 days and 1 day of treatment with 100, 200 mg vaginal tablets of olchrimazole and their therapeutic efficacy. Chemotherapy 28 (Suppl 1):43, 1982. 4. One day treatment of vulvovaginal candidiasis with 500 mg clotrimazole vaginal tablet compared with 3 day regimen of two 100 mg vaginal tablets daily. Summany Report of studies at six centers. Miles Medical Research Report. D82-101, December 23, 1983. 5. Treatment of vulvovaginal candidiasis with 500 mg clotrimazole vaginal tablet compared with a vehicle tablet. Summary report of multicenter study. Miles Medical Research Report D81-033, December 21, 1983. 6. Data on file, Medical Department, Miles Pharmaceuticals.

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CLINICAL SECTION

Clinical Opinion

Diagnostic tests in obstetrics: A method for improved evaluation

Douglas K. Richardson, M.D., M.B.A., J. Sanford Schwartz, M.D., Paul J. Weinbaum, M.D., and Steven G. Gabbe, M.D.

Philadelphia, Pennsylvania

With the proliferation of diagnostic tests in obstetrics, several recurrent questions arise. How does one determine whether one diagnostic test is superior to another available test? What test cutoff value best separates diseased from nondiseased patients? How much does performance of additional tests assist in arriving at a correct diagnosis? This article reviews a simple yet sophisticated analytic technique, the receiver operating characteristic curve, and demonstrates its application to several obstetric diagnostic tests. Receiver operating characteristic curve analysis is used to select a cutoff value for the 1-hour glucose tolerance test, to compare amniotic fluid tests of fetal lung maturity, and to determine the optimal combinations of factors and overall performance of the fetal biophysical profile. The value of receiver operating characteristic curve analysis lies in providing a clear graphic analysis of the performance of diagnostic tests over their entire range of values. It also provides the starting point for evaluating the costs and benefits of alternative cutoff points in differing clinical settings. (AM J OBSTET GYNECOL 1985;152:613-8.)

Key words: Antenatal diagnosis, fetal evaluation, decision analysis

Diagnostic tests play a central role in the practice of obstetrics. However, such tests pose several common problems for physicians. Which test cutoff values best separate diseased from nondiseased patients? How does one determine whether one diagnostic test is superior to an alternative test? How much does performance of additional tests assist in arriving at a correct diagnosis? Previous writers in this journal have discussed the basic concepts of sensitivity, specificity, and predictive value of diagnostic tests (Table I). In this article we will review the principles of a simple yet more sophisticated analytic technique, the receiver operating characteristic curve, which uses the concepts of sensitivity and specificity to assist the physician in answering

the questions posed above. In discussing this technique, we will use common diagnostic problems faced by the obstetrician and refer to data in the published obstetric literature. The receiver operating characteristic curve has been applied to similar situations in radiology, clinical chemistry, and internal medicine. As stated by Metz, "Although these concepts are (unfortunately) often clothed in seemingly occult jargon (because of the need for concise and precise terminology) the principles are mostly formalized common sense, or at least can be recognized as reasonable when explained in plain language." This article aims to present such a straightforward explanation to researchers and practitioners in obstetrics and gynecology.

The receiver operating characteristic curve is a simple concept with a complex name. It derives from signal detection studies of radar operators in the 1950s. Radar operators must distinguish a true signal from background noise. It was noticed that whenever an operator increased his detection of true signals, an increase in false alarms (false positives) also resulted. Thus there was a tradeoff between increasing detection of true

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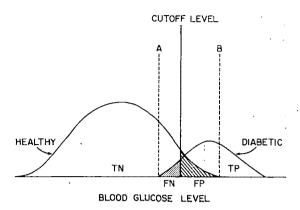


Fig. 1. Schematic diagram of the effect of glucose cutoff level on detection versus misclassification of gestational diabetes. FN = False negatives, FP = false positives, TN = true negatives, and TP = true positives.

signals (sensitivity) and minimizing false alarms (false positive rate). These tradeoffs all fell in a continuum along a curve, the receiver operator characteristic curve, which was a characteristic of the radar operator and the receiver. This curve defined the maximal resolution of the operator/radar device combination. The parallels with radiology were immediately evident: a radiographic image may be interpreted with the use of lenient or strict criteria, but the resolution can be improved only by changing the type of imaging equipment or the skill of the interpreter. This led to its adoption by Lusted for the evaluation of radiologic procedures.

Test result: call It positive or negative?

In screening for gestational diabetes, one may measure plasma glucose I hour following the ingestion of 50 gm of glucose (1-hour glucose tolerance test). How good is the 1-hour glucose tolerance test in discriminating between patients with and without gestational diabetes? What level of plasma glucose should be considered the top limit of "normal?" Conceptually, the 1hour glucose tolerance test attempts to separate diabetic from nondiabetic patients based on a single blood sugar value. The biologic variability within these two groups, however, results in overlapping values between the two groups (schematically portrayed in Fig. 1). Since the curves of 1-hour glucose tolerance test values for diabetic and nondiabetic patients overlap, it is not obvious where the cutoff point between positive and negative test results should be placed. An examination of Fig. 1 shows that if the 1-hour glucose tolerance test cutoff point is set anywhere between points A and B, some patients with gestational diabetes will not be detected, and some nondiabetic patients will be misclassified as diseased. Only with a cutoff point below A is one assured of detecting all cases of gestational diabetes. The

Table I. Definitions of measures of diagnostic performance

Mecsure	Definition
Sensitivity	Percent of cases detected True positive results
Specificity	True positive + false negative results = Percent of normal subjects classified correctly
	True negative results
False positive rate	True negative + false positive results Percent of normal subjects classified in- correctly
	False positive results
=	True negative + false positive results 1 - specificity

higher the 1-hour glucose tolerance test cutoff point is set, the more patients with true gestational diabetes would be misclassified as being nondiabetic (false negatives: dotted area, Fig. 1) but fewer normal patients would require follow-up retesting with a 3-hour glucose tolerance test (false positives: hatched area, Fig. 1). Conversely, the lower the cutoff point is set, the fewer cases will be missed, although more nondiabetic patients will be labeled as suspected to be diabetic. Whatever diagnostic test cutoff point is selected, four types of patient classifications are possible, which can be displayed in a two-by-two table (Table II).

From this table one can calculate the sensitivity (percent of diabetic subjects detected) and false positive rate (percent of normal subjects incorrectly classified as diseased) of the test. The obvious problem faced by the clinician is how to select the appropriate test cutoff point from among the many possible alternatives. This is achieved by weighing the benefits and costs (in terms of mortality, morbidity, and dollars) of the four diagnostic outcomes that are possible. Intuitively the appropriate location for the cutoff for a diagnostic test is determined by balancing the costs of false negative results against the costs of false positive results on the one hand and the benefits of timely detection of true positive and true negative results on the other. In this example, these are the frequency and morbidity of undetected gestational diabetes versus the frequency and inconvenience of unnecessary follow-up 3-hour glucose tolerance tests (see Fig. 1).

The most commonly accepted cutoff point between normal and abnormal results for a 1-hour glucose tolerance test was established by O'Sullivan et al.⁶ Using a plasma glucose level of 143 mg/dl (equivalent to whole blood glucose of 130 mg), O'Sullivan et al. reported detection of 79% of gestational diabetic patients (sensitivity) while misclassifying only 13% of nondiabetic ones (false positive rate). Recently Carpenter and Coustan⁷ noted that 100% sensitivity in detecting ges-

Table II. Two-by-two matrix of possible test results for a 1-hour glucose tolerance test

	Gestational diabetes present	Gestationai diabetes absent
Glucose tolerance test		
Positive	True positive (case detected)	False positive (false alarm)
Negative	False negative (case missed)	True negative (true normal)

tational diabetes could be achieved by lowering the cutoff level of plasma glucose from 143 to 130 mg/dl. This change, of course, increased to 20% the proportion of nondiseased patients who were falsely classified as diabetics and who had to undergo retesting with a 3-hour glucose tolerance test (false positive results).

The question faced by the clinician is how to select the proper cutoff value for a given clinical situation. The receiver operating characteristic curve of a diagnostic test is a graph of the range of tradeoffs possible between increased detection of diseased individuals (sensitivity) and increased misclassification of nondiseased patients (false positive rate). It plots the test's sensitivity versus its false positive rate as the cutoff point is varied. For historic reasons it uses the false positive rate rather than the more common specificity (which is simply 100% minus the false positive rate). At each cutoff point a two-by-two table similar to that in Table II can be constructed. Fig. 2 represents the receiver operating characteristic curve for the 1-hour glucose tolerance test based on the data of Carpenter and Coustan.⁷ This curve is unique for the 1-hour glucose tolerance test; the 1-hour postprandial glucose test or the 3-hour glucose tolerance test each would describe different curves. Note that the cutoff points used by O'Sullivan et al. and Carpenter and Coustan merely represent different points along the same receiver operating characteristic curve.

The receiver operating characteristic curve illustrates two important points. First, as one adopts a less stringent cutoff point in order to increase test sensitivity, the test's false positive rate also rises. Conversely, as one adopts a more stringent cutoff point for a diagnostic test, the false positive rate decreases but is accompanied by a concomitant decrease in sensitivity. The only ways both to increase test sensitivity and to decrease the false positive rate are either to improve the test itself (that is, develop a new, better assay, the "receiver") or to improve the skill of the test interpreter (the "operator"). This latter factor is particularly relevant when test interpretation skill plays an important role in test performance, as is common with many diagnostic tests (for example, electronic fetal heart rate monitoring). Second, the cutoff point selected always represents a trade-

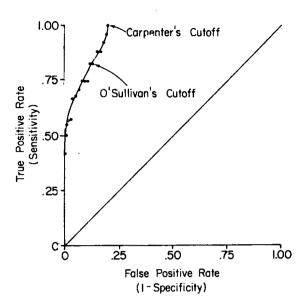


Fig. 2. Receiver operating characteristic curve of 1-hour plasma glucose test in detecting gestational diabetes. Data from Carpenter et al.7

off between true positive and false positive results. Thus the appropriate cutoff point depends on one's clinical judgment regarding several factors: the severity of the d sease if not treated; the availability, safety, and effectiveness of available treatments; the psychological and financial costs of treatment; the costs of unnecessary wo-kup or empirical treatment of nondiseased patients misclassified as diseased; and the prevalence of the disease in the population under study. It is possible that the same test should be used at different cutoffs in different clinical situations, such as in screening versus diagnostic use. A mathematical formula has been developed to permit one to calculate the appropriate operating point if one can estimate the above information_8

Comparison of alternative diagnostic tests

Receiver operating characteristic curve analysis is the optimal method to compare different diagnostic tests that are used for the same purpose. For example, there are numerous tests available for predicting fetal lung maturity: lecithin/sphingomyelin ratio,9,10 optical density at 650 nm,11,12 foam stability index (a commercial version of the shake test),13,14 phosphatidyl glycerol by thin-layer chromatography9 or rapid slide agglutination,10 and fluorescence polarization.16 Of particular clinical interest are the optical density at 650 nm and the foam stability index because of the ease and rapidity of their performance and thus their potential wide availability to clinicians in a large variety of practice settings. Yet the published literature has presented the sensitivity and specificity of these tests only at single cuto-f points. The published sensitivity and false pos-

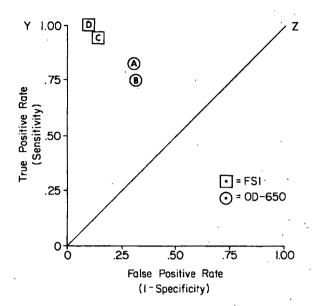


Fig. 3. Receiver operating characteristic plot of results of foam stability index and test of optical density at 650 nm selected from published studies. Data from (A) Copeland, ¹¹ (B) Khouzami et al., ¹² (C) Sher and Statland, ¹³ and (D) Lipshitz et al. ¹⁴ (see text).

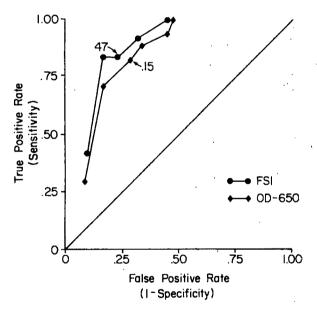


Fig. 4. Receiver operating characteristic curves of foam stability index and test of optical density at 650 nm. Data from Schwartz et al.¹⁷ with standard cutoff points marked.

itive rates at the standard cutoff points for these tests are plotted in Fig. 3. Although the foam stability index appears to have a higher sensitivity than the test of optical density at 650 nm, it is often not possible by visual inspection, at the single cutoff reported, to determine which of these two diagnostic tests is superior.

The only way to determine which test actually exhibits better diagnostic performance is to derive separate receiver operating characteristic curves by varying the cutoff point for each test, calculating the resultant

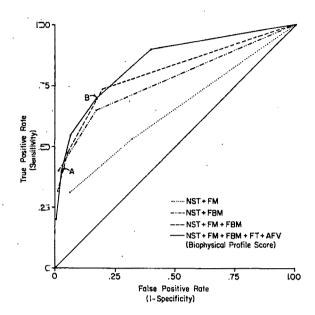


Fig. 5. Feceiver operating characteristic curve of partial and full biophysical profiles in predicting low 5-minute Apgar scores (\leq 7). Data from Manning et al. 20,21 NST = Nonstress test, FM = fetal movement, FBM = fetal breathing movement, FT = fetal tone, and AFV = amniotic fluid volume. A, B: Cutoff points for immediate action or 24-hour reevaluation respectively.

sensitivity and false positive rate at each point, and then plotting the results, as described above. A test with no diagnostic value (that is, random) would appear as a 45-degree line through the origin (line O-Z, Fig. 3). Each point on this line represents an equal likelihood of a true and false positive result as would occur by chance alone. A perfect test would ascend the y axis to point Y (100% sensitivity) without incurring a single false positive result. Such perfection rarely occurs. In general, one deals with imperfect tests whose receiver operating caaracteristic curves lie between point Y and the 45-degree line in Fig. 3. A superior diagnostic test exhibits a receiver operating characteristic curve above and to the left of another test's receiver operating characteristic curve. That is because each point on the upper receiver operating characteristic curve represents a higher test sensitivity for any given false positive rate or, conversely, a lower false positive rate for any given level of text sensitivity.

In Fig. 4 we present our own receiver operating characteristic curves for the foam stability index and test of optical der sity at 650 nm in predicting neonatal respiratory distress syndrome, with the conventional cutoff points marked. ¹⁷ Although the diagnostic performance of the foam stability index appears to be slightly better than that of the optical density test at 650 nm, are these differences real or are they due to chance? Methods of calculating the statistical significance of differences between curves have been developed. ^{18, 19} When applied

to the curves in Fig. 4, these methods confirm that there is no demonstrable difference between these two tests. Comparisons of the optical density at 650 nm and foam stability index assays with the lecithin/sphingomyelin ratio, however, reveal the lecithin/sphingomyelin ratio to be superior to both the optical density and foam stability index tests.17 Direct comparisons among the other tests mentioned9, 15, 16 could be easily derived from the raw data of the original studies.

Nonetheless, one may not necessarily always prefer a test with the best receiver operating characteristic curve performance. A test with an inferior receiver operating characteristic curve may be preferred if it is significantly faster, easier to perform, safer, or less expensive or if the test result will not significantly alter patient management or outcome. In clinical obstetrics, the foam stability index and the test of optical density at 650 nm are comparable tests which still may attain wide clinical use as rapid, readily accessible clinical tools when a lecithin/sphingomyelin test is not available or as relatively inexpensive screening procedures to eliminate the need for lecithin/sphingomyelin ratios in many obviously mature amniotic fluid samples.

Combination of tests

Clinical obstetrics generally requires collecting and integrating many pieces of data. Common problems are determining (1) whether or not to perform an additional test and (2) how to combine different diagnostic tests optimally. Receiver operating characteristic curve analysis is useful in determining the marginal impact of additional diagnostic testing. The mechanics are very similar to that of the use of receiver operating characteristic curves for individual tests. However, for combinations of diagnostic tests, one calculates the sensitivity and false positive rates of selected combinations of tests as one varies the cutoff points of the individual tests making up the combination.

Returning to the previous example of the foam stability index and the optical density test at 650 nm, one can pose the question of whether both tests used in combination might provide more accurate results (a higher receiver operating characteristic curve) than either test alone. Such test combinations can be used to attain higher sensitivity (either test positive) or a lower false positive rate (both tests positive).3

In another obstetric application of combination testing, Manning et al.20 recently reported a multivariate biophysical profile for predicting fetal compromise as demonstrated by low Apgar scores. The biophysical profile is simply the nonstress test plus several scaled measures of fetal tone and activity observed during ultrasound examination. Each scale can be considered a "test." By combining five such individual tests (each with high sensitivity but also with false positive rates of

>50%), Manning et al. derived a total score whose performance was markedly superior to that of any single test. The quantitative nature of the profile scores lends itself well to receiver operating characteristic curve analysis. In Fig. 5 we present receiver operating characteristic curves calculated from the published results of Manning et al. Note that additional "tests" improve the curve, but to a diminishing degree. Using this stepwise technique, test designers can identify which test components contribute the most diagnostic information and the marginal diagnostic information provided by additional tests, optimize the overall scale, and minimize the number of observations the clinician must obtain. Note also the smoothness of the curve, which illustrates that there is no obvious cutoff point; any cutoff point will miss some asphyxiated fetuses if excessive numbers of false positive results are to be avoided. The cutoff point for active intervention used by Manning et al. on a subsequent study21 is marked A, and that used for repeat testing in 24 hours is marked B. Interestingly, in the range where the test is likely to be relied upon (at cutoff A), the nonstress test plus fetal breathing movements alone perform as well as the full profile score; that is, the other profile items add no discriminatory power.

Comment

The value of receiver operating characteristic curve analysis lies in providing a clear graphic analysis of the efficacy of diagnostic tests over their entire range of values rather than at just a single cutoff point. A receiver operating characteristic curve also provides the quantitative method to determine the optimal cutoff point for a diagnostic test based on the costs of false positive and false negative results, the benefits of true positive and true negative results, and the prevalence of the disease. Thus the technique is useful for selecting an appropriate cutoff point in differing clinical situations. The receiver operating characteristic curve is an essential tool for comparing alternative diagnostic tests. Without it one cannot usually be certain that apparent differences in performance levels among diagnostic tests are not due solely to the use of different cutoff points by different investigators. Receiver operating characteristic curve analysis is also extremely useful in evaluating the utility of combination testing and in optimizing the information gained by the tests selected. Moreover, receiver operating characteristic curve analysis evaluates the complete test/interpreter unit. This is of particular importance in obstetrics where many tests demand a great deal of expertise to perform and interpret accurately. Furthermore, many tests and procedures are initially evaluated in highly specialized settings with much professional expertise and high-risk populations. Yet the tests must be applied in the more common setting of community practice. In such settings, test performance may be expected to decline somewhat from published results. Because of differences in technique, interpreter skill, and prevalence of disease, test cutoff points appropriate for one setting may not be appropriate for another. Thus, receiver operating characteristic curve analysis provides a method for determining what cutoff points to use and the level of performance one can expect in one's own practice setting. Increased use of receiver operating characteristic curve analysis should lead to improved evaluation and application of diagnostic tests and procedures.

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Clinical Articles

Safety of abortion and tubal sterilization performed separately versus concurrently

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Concurrent abortion and sterilization are preferred by many women to avoid a second hospitalization, operation, and, in some instances, general anesthesia. Several authors have shown concern, however, that the two procedures carry a higher risk of morbidity when performed concurrently versus separately. To determine whether the concurrent performance of sterilization and induced abortion is as safe as the two procedures performed separately, we selected women undergoing these procedures from two separate multicenter, prospective, national United States studies: the Joint Program for the Study of Abortion and the Collaborative Review of Sterilization. Using standard definitions of major morbidity, we calculated the crude rate of one or more major complications to be 0.9% for the abortion-only group, 1.7% for the group concurrent abortion and tubal sterilization. Thus our data suggest that performing concurrent abortion and sterilization is as safe as performing those procedures separately. (AM J OBSTET GYNECOL 1985;152:619-23.)

Key words: Abortion, sterilization, concurrent abortion and sterilization

Tubal sterilization and legal induced abortion are among the most frequent surgical procedures performed on women aged 15 to 44 in the United States. About 650,000 tubal sterilizations and 1,157,776 legal induced abortion procedures were performed in 1978. ¹¹² Of these procedures, 22,000 (3.4%) tubal sterilizations and induced abortions were performed concurrently.¹ The increased demand for postabortion tubal sterilizations has led to debate over the safety of combining the two procedures.

Previous reports have suggested that morbidity and mortality rates associated with tubal sterilization may be increased by performing the procedure at the time of abortion, possibly as a result of physiologic changes during pregnancy.^{3,4} However, a review of studies done before 1978 revealed no evidence of increased complication rates for women having a tubal sterilization at the time of induced abortion,⁵⁻¹¹ although most of

those studies did not directly study the question of the comparative safety of abortion and tubal sterilization performed separately versus concurrently.

Among these, some studies^{7, 10} have compared complication rates associated with the combined procedures (tubal sterilization and abortion) with those of abortion alone or with those of tubal sterilization alone.¹¹ Furthermore, comparison of morbidity between these studies is made more difficult because of the use of different definitions⁸ and the lack of control for potentially confounding variables in the analyses of most of the studies.

In this study, we compared the complication rates associated with tubal sterilization and abortion performed concurrently with the complication rates of the procedures performed separately. Using standard objective criteria for complications, 12 we compared complication rates for women in groups having the following procedures: (1) first-trimester abortion only, (2) concurrent tubal sterilization and first-trimester abortion, and (3) tubal sterilization not associated with pregnancy.

Methods

Data collection. For calculating complication rates for abortion alone, tubal sterilization alone, and for the

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Table I. Percentage distribution of selected demographic characteristics of women undergoing abortion and tubal sterilization separately and concurrently

•	JPS	JPSA		REST
	Abortion alone (n = 14,029)	Concurrent* (n = £93)	Tubal sterilization alone† (n = 4925)	Concurrent (n = 183)
Age (yr)			•	
20-29	78.2	37.9	41.3	45.4
30-44	21.8	62.1	58.7	54.6
Race	•			
White	, 44.0 .	39.G	65.8	56.8
All other races	56.0	60.1	34.2	43.2
Marital status				
Never married	51.2	14. <u>Ç</u>	14.1	14.8
Ever married	48.8	85.1	85.9	85.2
No. of previous pregnancies				
· ≤ 2	69.7	27.9	44.8	12.6
>2	30.3	72.1	55.2	87.4
Presence of preexist- ing medical condi- tions	10.1	21.4	31.9	40.4
Board-certified obste- trician/gynecologist	66.7	47.1	74.2	72.7
Approach to the tube	4 · · · · · · · · · · · · · · · · · · ·			
Laparotomy	. N/A\$	15.3	8.0	9.8
Other‡	· N/A§	84.7	92.0	90.2

^{*}Concurrent tubal sterilization and legal induced abortion.

concurrent abortion and tubal sterilization procedures, we used data from two separate multicenter, prospective studies in the United States: the Joint Program for the Study of Abortion (JPSA) and the Collaborative Review of Sterilization (CREST).

JPSA is the study of early complications of legal induced abortion conducted under the auspices of the Centers for Disease Control (CDC). Between 1975 and 1978, 13 institutions provided information on approximately 84,000 induced abortions. Data were collected from university hospitals and proprietary clinics. We eliminated abortions performed at proprietary clinics in JPSA to make operators' experience more comparable between the two studies, since tubal sterilization data in CREST were available for hospitals only. We also excluded abortions performed on women aged 19 years or less. These exclusions limited our analysis to 14,922 women aged 20 to 44 years, 14,029 of whom had a first-trimester abortion and 893 of whom had a first-trimester abortion with concurrent tubal sterilization.

CREST, which began in 1978, was designed to assess the safety and efficacy of various tubal sterilization procedures. Women were enrolled from nine centers in five cities. We selected 4925 women aged 20 to 44 years who had tubal sterilization alone and 183 women who

had a tubal sterilization concurrent with first-trimester abortion in the time period 1978 to 1981. The tubal sterilization procedures included all the approaches to the fallopian tubes; methods of tubal occlusion included electrocoagulation, bands, clips, fimbriectomy, and salpingectomy.

We determined complication rates for four groups of women: (1) the JPSA abortion-alone group—women in the JPSA study who had had a first-trimester abortion at 12 weeks' gestation or less (n = 14,029); (2) the CREST tubal sterilization—alone group—women in the CREST study who were not pregnant at the time of their hospital admission for a tubal sterilization (n = 4925); (3) the JPSA concurrent group—women in the JPSA study who had had a first-trimester abortion together with a tubal sterilization (n = 893); (4) the CREST concurrent group—women in the CREST study who had had a first-trimester abortion by vacuum aspiration along with the sterilization procedure (n = 183).

Definition of complications. We used the following definitions of intraoperative and postoperative, in-hospital complications: (1) unintended major surgical procedure—any laparotomy, repair of a perforated viscus, or repair of a major blood vessel that was performed intraoperatively or postoperatively during the same

[†]Tubal sterilization not associated with pregnancy.

[‡]Includes approximately 5% culdoscopic procedures (vaginal approach).

[§]Not applicable.

Table II. Crude complication rates* of one or more complications by study group

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Complication	Abortion alone (n = 14,029)	Concurrent ⁺ (n = 893)	Tubal sterilization alone (n = 4925)	Concurrent $(n = 183)$
Fever >2 days	0.3	1.0	0.6	0.6
Transfusion .	0.1	0.1	0.0	0.0
Unintended surgical procedure	0.6	1.4	1.2	1.1
Life-threatening event	0.0	0.0	0.1	0.0
Death	0.0	0.0	0.0	0.0
One or more complications	•			
Crude rate	0.9	2.1	1.7	1.6
Operator training— adjusted rate	0.9	1.7	1.8	1.7

^{*}Rate per 100 women.

hospitalization, that was not planned, and that was necessary because of a problem related to the tubal sterilization or abortion; (2) transfusion—any intraoperative or postoperative blood transfusion; (3) febrile morbidity—oral temperature ≥38.0° C during at least 2 postoperative days, excluding the first 24 hours after operation; (4) other life-threatening events—any intraoperative or postoperative cardiac or respiratory arrest, myocardial infarction, pulmonary embolus, anaphylactic shock, or disseminated intravascular coagulation; (5) death—death or complication leading to death occurring within 42 days of operation.

These categories of complications were chosen because they represent life-threatening events or could reasonably be expected to result in increased morbidity. Moreover, it is reasonable to make comparisons between different institutions and different surgical procedures because these complications are objective, clearly defined outcomes that are not subject to much variability in interpretation.

Since CREST follow-up data were collected by both chart review and telephone interview and JPSA data by chart review only, the completeness of follow-up data concerning rehospitalization in both data sets was not comparable. We, therefore, excluded rehospitalization from our complication criteria and restricted our analysis to in-hospital complications.

Analytic technique. We calculated complication rates for each of the groups separately. To determine which variables produced confounding in our data, we calculated the crude risk of having one or more complications for abortion alone versus concurrent tubal sterilization and abortion using JPSA data. We also calculated the crude risk of complications for tubal sterilization alone versus concurrent tubal sterilization and abortion using CREST data. We then standardized for

potentially confounding variables using the Mantel-Haenszel¹³ method. We examined several clinically important characteristics to determine whether important differences between our study groups existed. Specifically, the variables we examined included education level, age, gravidity, and marital status of the patient; level of operator training, preexisting medical conditions, and approach to the fallopian tubes. Of these, only level of operator training was a confounding variable in our data set. To allow comparison of the different group complication rates, we directly standardized them for level of operator training (Board-certified obstetricians/gynecologists versus other physicians in training). We then determined the relative risk and 95% confidence interval of complications for the CREST concurrent group versus the CREST alone group using methods indicated by Rothman and Boice.14

Results

Among the four groups, women in the abortionalone group tended to be considerably younger, be ever married, and report fewer prior pregnancies than women in the other groups (Table I). However, controlling for age, marital status, and gravidity by the Mantel-Haenszel method did not appreciably alter our results. Both concurrent and tubal sterilization—alone groups from the CREST study had proportionately more white women than either of the JPSA groups.

Women who had tubal sterilization alone or concurrently with abortion were more likely to have a preexisting medical condition than women in the abortionalone group (Table I). Women in the CREST groups were more likely to have their procedures performed by Board-certified gynecologists than women in the JPSA groups. The tubal sterilization procedures were mainly performed via the laparoscopic approach. The

[†]Concurrent tubal sterilization and legal induced abortion.

crude complication rates for one or more complications were 0.9% for the abortion-alone group, and 2.1% for the JPSA concurrent group (Table II). The crude complication rates were 1.7% for the tubal sterilization—alone group and 1.6% for the CREST concurrent group.

The complication rate adjusted for the level of operator training was 0.9% for the JPSA abortion-alone group, 1.7% for the JPSA concurrent group, 1.8% for the CREST tubal sterilization—alone group, and 1.7% for the CREST concurrent group.

Analyzing the CREST data only, we found that women having concurrent tubal sterilization and induced abortion had a risk of developing one or more complications similar to that of women having tubal sterilization alone (operator-adjusted relative risk = 1.0, 95% confidence interval 0.3 to 3.0).

Comment

Our data show for the study population that the risk of complications for women having concurrent tubal sterilization and induced abortion is similar to that for women having tubal sterilization alone. This is intuitively difficult to reconcile with data demonstrating that abortion alone and tubal sterilization alone carry certain morbidity risks. 12, 15 Two possible explanations are: (1) The CREST concurrent tubal sterilization and abortion group was relatively small. Thus by chance alone the CREST concurrent tubal sterilization and abortion complication rate may be similar to that of the tubal sterilization alone group. That the complication rate for the JPSA concurrent group was of a similar magnitude to that of the CREST concurrent group, however, lends support to the contention that there is little difference between the complication rates for the concurrent groups versus the tubal sterilization-alone group. (2) Although a separate complication may have occurred with each procedure (tubal sterilization and abortion), for the concurrent group such complications would likely be listed as a single complication, whereas for the procedures performed separately they would be reported as two separate complications. To the extent that this occurred, the complication rate for the concurrent group is low.

Although this is the largest reported study examining this issue, it has the following limitations. The data came from two different studies and were collected by different interviewers over different time periods and from different institutions. The CREST data in our study were collected between 1978 and 1981 from nine hospitals located in five United States cities. All nine hospitals were affiliated with university medical centers. Data were collected both by chart review and by patient interview conducted by trained personnel. By contrast, JPSA data were obtained from nine hospitals from all

regions of the country. Data were collected primarily by chart review.

To limit ascertainment bias that might result from comparing data from the two studies, we only considered major, objective complications documented in medical charts. As there were differences in the method of determining rehospitalization for the two studies, we did not analyze rehospitalization as a separate complication. The similarity of the complication rates for the JPSA and CREST concurrent groups, however, suggests that this did not occur to a large extent.

Based on the available data, we defined women who had tubal sterilization alone as all those who were not pregnant at the time of hospitalization, whereas most other studies used the term "interval" to define a tubal sterilization performed at least 6 weeks after the termination of the last pregnancy. Thus we included women in the tubal sterilization—alone group who never obtained an abortion and those who obtained an abortion recently but not during this hospitalization for a sterilization procedure. Inclusion of women who had had a recent abortion and potentially had a greater risk of complication after tubal sterilization would increase the complication rate in our tubal sterilization—alone group. It is unlikely that this occurred to a substantial degree.

Since our definitions of major complications differ from those definitions used in other studies, we cannot make a direct comparison of our results with specific complication rates reported in other studies examining this issue. Some studies included separate rates for major and minor complications, immediate and delayed complications, early complications, and immediate and early complications. Nevertheless, the relative risks of complications for the procedures performed separately versus concurrently can be compared if it is assumed that the same definitions of complications were used for comparison groups with each study.

Short-term morbidity is one of a number of issues requiring consideration when the safety of combined abortion and tubal sterilization is discussed. Our study supports earlier findings that there is no increase in immediate morbidity following a concurrent procedure as opposed to separate abortion and tubal sterilization procedures.

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Cesarean section deliveries among adolescent mothers enrolled in a comprehensive prenatal care program

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A comparison was made of the incidences of cesarean section deliveries between mothers 18 years of age or younger and those 19 to 21 years of age, all of whom were enrolled in a comprehensive program of prenatal care. Also compared were the indications for surgical delivery between the two groups. The rate of cesarean section deliveries was 8.7% among mothers 18 years of age or younger, compared to 14.5% among mothers 19 to 21 years of age. The general-ward cesarean section rate at the same hospital was 19.4%. Since maternal height and weight, and infant's birth weight may affect the diagnosis of cephalopelvic disproportion, these parameters were also evaluated. However, these did not differ significantly between mothers 18 years of age or younger and those 19 to 21 years of age or between vaginal and surgical births in mothers 18 years of age or younger. Thus, cephalopelvic disproportion did not contribute importantly to the cesarean section rate. (AM J OBSTET GYNECOL 1985;152:623-6.)

Key words: Cesarean section, adolescent pregnancies, comprehensive prenatal care

Cesarean section birth rates for childbearing women of all ages have increased in the United States during the past two decades. In nonfederal short-stay hospitals, this rate rose from 4.5% in 1965 to 17.9% in 1981. Numerous reasons have been cited for this increase, including the fact that, recently, more women over 30 years of age have been giving birth. Since these older women have a greater chance of complications, they may experience higher rates of cesarean section deliveries. However, the rate of cesarean section births

among adolescent mothers also increased from 3.1% in 1965 to 13.2% in 1981. Although these rates are lower than those among older women, they represent a substantial increase for this age group.

Information in regard to indications for cesarean section births among adolescent mothers is not particularly consistent. Several studies conducted during the 1960s suggested that cesarean section deliveries among teenagers, particularly the very young, were primarily related to the relatively high incidence of cephalopelvic disproportion in this age group. However, in 1970, Coates Lound no differences between teenagers older than 14 years and those younger in the incidence of contracted pelvis, abnormal presentation, or uterine dysfunction. Moreover, the cesarean section birth rates in these two groups did not differ significantly. Similarly, Perkins et al. failed to find significant differences between teenagers and older women in the incidence of cephalopelvic disproportion or cesarean section

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Table I. Percentage of cesarean section births, by age

Age of mother (yr)	Percentage of cesarean section births
15 or under (N = 97)	9.3
16-18 (N = 636)	8.6
19-21 (N = 896)	14.5
Total ($N = 1629$)	11.9

births. Interestingly, Spellacy et al.⁹ found that the frequencies of abnormal presentation, fetal distress, placenta previa, abruptio placentae, and prolapsed cord among teenagers were equal to or greater than those among mature women. However, cephalopelvic disproportion was not more prevalent among teenagers, and the rates of cesarean section births were similar in teenagers and older women. On the other hand, one study found that girls 15 years of age or younger had pelvic inlets that were significantly smaller than those of older women, but that the cesarean section rates for teenagers and for women who were 19 to 25 years of age did not differ significantly.¹³

More recently, one institution reported cesarean section rates of 33% for 14-years-olds, 24% for 15-year-olds, and 18% for 16-year-olds, but did not report the indications for cesarean section births. In another study, the cesarean section rate for adolescent mothers in the general clinic was 14.7%. This rate was 9.2% for mothers of similar age in a specialized program, despite a larger mean birth weight among this group. Also, cephalopelvic disproportion was the least common indicator for cesarean section delivery among these young mothers.

The purpose of our report is twofold. First, we compare the incidences of cesarean section deliveries between mothers 18 years of age or younger and those 19 to 21 years of age, all of whom were enrolled in a comprehensive program of prenatal care. Second, we compare the indications for surgical delivery between the groups.

Methodology

All young mothers who had cesarean section deliveries between September, 1980, and June, 1983, and were enrolled in the comprehensive prenatal program of the Division of Perinatology of the Departments of Obstetrics and Gynecology and Pediatrics, Columbia University, were included in this evaluation. The program is staffed by certified nurse-midwives, obstetric residents, and attending obstetricians who specialize in maternal-fetal medicine. Its purpose is to provide comprehensive antenatal care adapted to the needs of the adolescent population in the Washington Heights community of Manhattan, New York. ¹⁵ All pregnant young

women who are under 21 years of age are enrolled in this comprehensive program, no matter when in the course of their gestation they register for care. The staff provides care based on philosophy described elsewhere. 14, 15

All of the young mothers included in this study were managed during their labor by a resident in obstetrics in consultation with an attending member of the Department of Obstetrics and Gynecology. A cesarean section was performed only when a chief resident and the attending physician agreed that it was necessary. During the study period, the general policy of the resident's service was to deliver by repeat section those women who had had previous cesarean sections.

Electronic fetal monitoring was carried out in all of the young mothers during their labor, unless they were in such active labor that they delivered very shortly after their arrival at the hospital. The diagnosis of fetal distress was made on the basis of fetal heart rate patterns in conjunction with fetal scalp sampling, with the use of standard definitions and treatment carried out according to the protocols of the Division of Perinatology. Young mothers with breech presentations, including primigravid mothers, were allowed a trial of vaginal labor if the vertex was well flexed, the estimated fetal weight was between 2500 and 4000 gm, and x-ray pelvimetry disclosed adequate pelvic diameters. Cesarean section was the method of delivery in breech presentations when the above-mentioned criteria were not satisfied or when labor was judged to be dysfunctional. Pitocin induction and augmentation were conducted as indicated by standard obstetric protocol. Cesarean section for failure to progress or arrest of labor was not resorted to unless an adequate trial of Pitocin was unsuccessful. Cephalopelvic disproportion was always diagnosed on the basis of clinical pelvimetry, and in approximately one half of the cases the diagnosis was supplemented by x-ray pelvimetry. In addition, all cases that needed surgical delivery were presented to the clinical chief of service for review, and the indication for such a delivery was discussed.

Information in regard to cesarean sections was collected from statistical records kept by the certified nurse-midwives, from individual patient charts, and from data collected from birth registrars which are reported in the Monthly Sloane Hospital for Women Statistical Review. Indications for cesarean section deliveries were obtained from physicians' notes and from the intrapartum records of the hospital.

Since maternal height and weight may be related to cephalopelvic disproportion, heights, and weights were recorded from the initial antenatal records and were checked for accuracy by comparing these measurements with records of previous visits. The prepregnant heights and weights of a sample of adolescent mothers

Table II. Frequency distribution of indications for cesarean section, by age of mother

	Ag= (yr) of mother				
	18 or younger		19 to 21		
Indication for cesarean section	No.	%	No.	%	
Repeat cesarean section	18	25.0	35	39.8	
Fetal distress	17	23.7	21	23.9	
Abnormal presentation	14	19.4	8	9.1	
Cephalopelvic disproportion	8	11.1	11	12.5	
Failure to progress	10	13.9	8	9.1	
Arrest of labor	5	6.9	2	2.3	
Abruptio placentae	0	0.0	1	1.1	
Prolapsed cord	0	0.0	1	1.1	
Severe preeclampsia	_0	0.0	1	1.1	
	$\overline{72}$	(100.0)	$\overline{88}$	(100.0)	

Table III. Prepregnant maternal heights and weights and infants' birth weights among mothers having cesarean births, by age

	Maternal age (yr)		
	18 or younger	19 to 21	
Prepregnant weight	1		
Mean*	123.98	124.39	
Standard deviation	24.33	20.47	
Standard error	3.75	2.43	
N	42	71	
Prepregnant height			
Mean*	62.03	61.69	
Standard deviation	2.49	3.48	
Standard error	0.41	0.46	
N	37	57	
Infant birth weight			
Mean*	3065	3258	
Standard deviation	718	639	
Standard error	107	72	
N	45	78	

^{*}Not significant by Student's two-tailed t test.

18 years of age or younger who had vaginal deliveries were also recorded. These data allowed a comparison between young mothers who had vaginal deliveries and those who had cesarean section deliveries. These data were analyzed by means of Student's two-tailed t tests.

Findings

During the evaluation period, the rate of cesarean section deliveries was 11.9% among mothers 21 years of age or younger who were enrolled in the comprehensive prenatal program, compared to a general-ward cesarean rate of 19.4%. As can be seen in Table I, the percentage of cesarean section deliveries was 9.3% among mothers 15 years of age or younger, compared to 8.6% among mothers 16 to 18 years of age and 14.5% among mothers 19 to 21 years of age.

Table II presents the frequency of indications for cesarean section among 160 young mothers who had reasons clearly documented in their records. The rec-

Table IV. Prepregnant maternal heights and weights among mothers 18 years of age or younger, by type of delivery

	Type of delivery		
	Cesarean section	Vaginal birth	
Prepregnant weight			
Mean*	123.98	125.80	
Standard deviation	24.33	20.71	
Standard error	3.75	3.09	
N	42	45	
Prepregnant height			
Mean*	62.03	62.34	
Standa-d deviation	2.49	3.24	
Standa-d error	0.41	0.51	
N	37	41	

^{*}Not significant by Student's two-tailed t test.

ords of 34 of the 194 patients (17%) were either inadequate for this assessment or could not be located during the study. The two most common indications among beth age groups were previous cesarean section and fetal distress. Interestingly, the obstetric complications of abruptio placentae, prolapsed cord, and preeclampsiz were very uncommon indications for cesarean section.

In Table III, the mean prepregnant heights and weights are compared between adolescent mothers 18 years of age or younger and mothers 19 to 21 years of age. There were no significant differences in height and weight between these two groups. This is not surprising, since cephalopelvic disproportion was a less frequent indication for cesarean section among the younger mothers than it was among the older ones. Similarly, birth weights of the infants of these mothers did not differ significantly.

When the prepregnant heights and weights of a sample of mothers 18 years of age or younger who were enrolled in the same program and who were delivered vaginally were compared to those of mothers of similar age who were delivered by cesarean section, again no significant differences were found (Table IV).

Comment

In this study, age was not an important factor in relationship to cesarean section birth. A comparison of mothers 18 years of age or younger to those 19 to 21 years of age, all of whom received the same comprehensive prenatal care given in the same environment and by the same professional staff, yielded insignificant differences in the rate of surgical births. The overall rate of cesarean section births among women 21 years of age or under in this comprehensive prenatal care program was 11.0%. This is considerably lower than the rates reported by Finkelstein et al.11 and by Placek et al.1 The rate reported here is similar to the one found by Neeson et al.12 (9.2%), and supports their study in also suggesting that comprehensive prenatal care for adolescent mothers may contribute to lowering the cesarean section birth rate among them.

Repeat cesarean section was the most frequent indication for surgical birth among both older and younger adolescent mothers, thus indicating that a previous cesarean section is a major risk factor that increases with age. This risk factor is iatrogenic, since, if these young mothers had been allowed a trial of vaginal delivery after a previous cesarean section, the surgical birth rate may have been even lower. Presently, in keeping with current obstetric practice, adolescent mothers in the program may be allowed a trial of labor after a previous cesarean birth.

For mothers under 19 years of age, cephalopelvic disproportion was the fifth most common reason for cesarean section. Interestingly, the frequency of this diagnosis was somewhat lower than it was among mothers 19 to 21 years of age. The mean maternal heights and weights and infant birth weights of patients 18 years of age or younger compared to those 19 to 21 years of age, were not statistically significant. However, the mean birth weight of babies born to mothers 18 years of age or younger was 133 gm less (Table III) than the mean birth weight of the babies of mothers 19 to 21 years of age. This difference could contribute clinically to the frequency of cephalopelvic disproportion in the younger group of mothers. In the population studied, we previously determined that 4% of younger mothers had babies of 32 weeks' gestation or less, compared to 1% of older mothers, a finding that partially explains the decreased mean birth weight of the infants born to younger mothers.13 In addition, maternal heights and weights were similar for patients 18 years of age or younger who had vaginal or surgical births. These are not particularly surprising findings, since cephalopelvic disproportion is a relative term and implies that a vertex is too large for the particular pelvis through which it must pass during birth.

Although we cannot be absolutely certain that the categories of failure to progress in labor and arrest of labor did not contain patients who in fact had cephalopelvic disproportion, it should be noted that the chief of service emphasizes the clinical differences between cephalopelvic disproportion and failure to progress, and arrest of labor caused by anomalies of the expulsive forces, whether they be uterine or those of the voluntary muscles during the second stage of labor. Thus, insofar as we are able to ascertain, patients assigned to the categories of failure to progress and arrest of labor had difficulties with the expulsive forces in general, rather than cephalopelvic disproportion.

In conclusion, then, in this study, cephalopelvic disproportion was not an important contributor to the cesarean section rate among young mothers, a finding that is in agreement with that of Spellacy et al.9

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Association of elective repeat cesarean delivery and persistent pulmonary hypertension of the newborn

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Seventy-one cases of persistent pulmonary hypertension of the newborn have been reviewed in an attempt to identify possibly preventable causes. Three groups of infants were identified. The first group consisted of 36 infants with evidence of perinatal asphyxia. The second group was made up of 23 infants who exhibited a variety of associated factors including pneumonia, septic shock, and congenital diaphragmatic hernia. A third group included 12 infants delivered by elective repeat cesarean section. Infants in the third group did not have evidence of perinatal asphyxia, meconium aspiration, or infection. Chest roentgenograms revealed amniotic fluid aspiration in seven cases, retained lung fluid in three cases, and normal findings in two cases. All 12 infants in the third group developed respiratory distress which eventually progressed to respiratory failure and persistent pulmonary hypertension of the newborn. These data suggest that infants of elective repeat cesarean deliveries are at risk for developing persistent pulmonary hypertension of the newborn and constitute a group of patients with a potentially preventable course of events. (Am J Obstet Gynecol 1985;152:627-9.)

Key words: Cesarean section, newborn infant, pulmonary hypertension

Since first described by Gersony et al.1 in 1969, persistent pulmonary hypertension of the newborn (also known as persistent fetal circulation) has been recognized as a serious problem occurring in the immediate newborn period. The clinical picture develops primarily in term and postterm infants in the first 24 hours of life and consists of evidence of pulmonary arterial hypertension with right-to-left shunting at the foramen ovale and/or ductus arteriosus and severe hypoxemia. During the last 15 years, a variety of perinatal complications have been determined to be associated with the occurrence of this disease; among them are perinatal asphyxia, aspiration syndromes, retained lung fluid, intrapartum infection, and congenital diaphragmatic hernia.2-5 Despite numerous treatment modalities, mortality remains 50% and higher. Obstetricians opting to identify antepartum events leading to the development of persistent pulmonary hypertension of the newborn may be able to prevent or ameliorate this disease process.5

In this study we identified the perinatal risk factors related to persistent pulmonary hypertension of the newborn in a series of infants in an attempt to uncover potentially preventable cases. We describe a group of infants, all of whom were products of elective repeat cesarean delivery, that appear to be at particular risk for persistent pulmonary hypertension of the newborn.

Subjects and methods

The 1257 newborn admissions during a 30-month period (July, 1980, to December, 1982) to the University of Kentucky Medical Center, Neonatal Intensive Care Unit, were reviewed to determine which infants had a clinical course consistent with persistent pulmonary hypertension of the newborn. Our diagnostic criteria for persistent pulmonary hypertension of the newborn are listed in Table I.

The cases were evaluated to ascertain which could have been potentially preventable. The prenatal course was reviewed for the following information: (1) length of labor prior to delivery, (2) presence or absence of ruptured membranes, (3) presence or absence of meconium-stained amniotic fluid, (4) evidence of fetal distress, (5) evidence of maternal infection, and (6) results of testing for fetal lung maturity. The intrapartum period was reviewed for: (1) type of anesthesia procedure used and (2) whether forceps were required. The postpartum period was reviewed for: (1) 1- and 5-minute Apgar scores, (2) evidence of infection, (3) length of time on ventilation, and (4) chest roentgenographic findings.

The chest roentgenograms were read by a pediatric radiologist without prior knowledge of the intent of the study. A diagnosis consistent with aspiration was made on the basis of a typical chest roentgenogram pattern

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Table I. Diagnostic criteria for persistent pulmonary hypertension

- 1. Arterial-alveolar gradient >500 torr with ${\rm Pa_{CO_2}}\!<\!40$ torr while on mechanical ventilation
- 2. Evidence of right-to-left shunt by Physical examination Differential oxygenation or oximetry Echocardiography
- 3. Gestational age ≥37 wk

Table II. Related diagnosis

Diagnosis	No. of . patients
Meconium aspiration	20
Perinatal asphyxia	16
Elective repeat cesarean delivery	12
Pneumonia	12
Sepsis	9
Congenital diaphragmatic hernia	2

of lung hyperinflation with irregular pulmonary densities and multiple streaks. The diagnosis of retained lung fluid was determined by a roentgenographic pattern which included fluid in the minor fissure and prominent perihilar streaking. A roentgenographic diagnosis of pneumonia was dependent on the appearance of a pattern of patchy infiltrates and/or air bronchograms.

Results

Seventy-one infants met diagnostic criteria for having persistent pulmonary hypertension of the newborn. Three groups of patients were identified. Group 1 consisted of 36 infants (51%) that had evidence of perinatal asphyxia. Of these, 20 infants were noted to have had a history of meconium aspiration that progressed to persistent pulmonary hypertension of the newborn. Of these 20 cases, 13 did not have tracheal suctioning performed prior to the infant's first breath. Group 2 consisted of 23 infants (32%) who had several associated factors including sepsis, pneumonia, and congenital diaphragmatic hernia (Table II). Group 3 consisted of 12 infants (17%) who were products of elective repeat cesarean delivery.

Thirty-one cases were the product of cesarean delivery with 15 cases classified as emergency deliveries and 16 cases as elective deliveries. Of the elective cesarean deliveries, four were primary deliveries, being performed for either breech presentation (three cases) or maternal interest (one case). The remaining 12 cases were elective repeat cesarean deliveries and were examined in greater detail (Table III). In none of the cases was an amniocentesis performed for fetal lung maturity testing, nor was fetal heart rate monitoring performed. Onset of labor was noted in two of the 12 cases, and ruptured membranes occurred in one case

Table III. Characteristics of elective repeat cesarean deliveries in 12 cases of persistent pulmonary hypertension of the newborn

- Parameter	No. of patients
Amniocentesis	0
Fetal distress	0
Labor	2
Ruptured membranes	1
Meconium	0
Asphyxia	
Apgar score <5 at 1 min	0
Apgar score <7 at 5 min	0
Presence of respiratory distress	
Birth	0
4 hr of age	4
8 hr of age	12
Evidence of infection	0
Chest roentgenogram findings	
Amniotic fluid aspiration	7
Retained lung fluid	3
Normal	3 2
Pneumonia	0
Length of ventilation	
112-300 hr (mean = hr)	11
650 hr	1
Air leak	6
Chronic lung disease	3
Death	3

2 hours prior to delivery. Meconium-stained amniotic fluid was not present in any of the cases and Apgar scores revealed no evidence of perinatal asphyxia.

All 12 infants were bulb-suctioned immediately after delivery and no further resuscitative efforts were required. Oxygen supplementation was not required in any of the 12 elective repeat cesarean deliveries at birth, but respiratory distress was present in four infants by 1 hour of age and was evident in all 12 infants by 8 hours of age.

No known signs of maternal infection occurred prior to, or after, delivery in the elective repeat cesarean section cases, and all routine studies for bacterial infection were negative.

Review of initial chest roentgenograms revealed findings consistent with amniotic fluid aspiration in seven cases. Findings consistent with retained lung fluid were noted in three infants and two had normal films. None of the infants was diagnosed as having congenital pneumonia.

All 12 infants required mechanical ventilation by 24 hours of age. Length of time on mechanical ventilation for the 10 survivors ranged from 112 to 300 hours (mean 176 hours) for nine infants with one infant requiring 650 hours of mechanical ventilation. Six of the 12 infants developed pneumothorax or other pulmonary air leak. Chronic lung disease of infancy (bronchopulmonary dysplasia) ultimately developed in two cases.

Three of the 12 infants died, two in the acute period

(at 3 and 4 days of age) and the third at 6 months of age after the development of cor pulmonale secondary to chronic lung disease of infancy. The nine survivors have been followed up in an outpatient setting for 18 to 48 months. One child exhibits abnormalities by the Denver Developmental Screening Test at 2 years of age, while the other eight children have no abnormalities on screening examination.

Comment

Data in this study are consistent with previous reports of the association of persistent pulmonary hypertension of the newborn and elective repeat cesarean delivery. Leder et al.,6 in 1980, reported that 15% of their patients with persistent pulmonary hypertension of the newborn were the product of elective repeat cesarean delivery. Schreiner et al.7.8 have also reported the association of amniotic fluid aspiration and retained lung fluid with the development of persistent pulmonary hypertension of the newborn in elective repeat cesarean deliveries.

National Institutes of Health statistics9 document that cesarean deliveries account for almost 20% of all deliveries, and elective repeat cesarean deliveries make up approximately one third.

Our studies revealed that 17% of all infants with persistent pulmonary hypertension of the newborn coming into our care were products of elective repeat cesarean deliveries and presented with chest roentgenographic patterns consistent with either amniotic fluid aspiration or retained lung fluid.

An absence of labor in most of the deliveries in these 12 cases may be associated with the subsequent development of respiratory distress and persistent pulmonary hypertension of the newborn. A number of possible explanations have been offered and they include (1) absence of labor-stimulated lung fluid resorption, 10 (2) lack of labor-stimulated lung maturation and surfactant release,11 and (3) lack of labor-induced diminution of pulmonary vascular resistance.12 Presently no clear etiologic explanation of persistent pulmonary hypertension of the newborn exists, and thus an explanation in relation to elective repeat cesarean deliveries cannot be offered as well.

In conclusion, it would appear from our observations that the infants who are products of elective repeat cesarean deliveries may do well initially only to be found later to have progressive respiratory distress and persistent pulmonary hypertension of the newborn. In the absence of a clearer understanding of the relation of persistent pulmonary hypertension of the newborn and elective repeat cesarean delivery an anticipatory 24hour period of special care and observation for these newborn infants appears warranted.

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Thiazide diuretics and bone mineral content in postmenopausal women

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This retrospective study of 54 postmenopausal women taking thiazide diuretics found that bone mineral measurements and bone fracture prevalence did not differ significantly from those of matched control subjects. Matching on the variables of type of menopause (surgical/nonsurgical), years postmenopausal, duration of estrogen therapy (if any), daily intake of dietary and supplemental calcium and vitamin D, and Quetelet index was done without knowledge of the bone mineral measurements. Bone mass was recorded as the bone mineral content and bone density of the distal and midshaft radius. Only fractures associated with osteoporosis (hip, rib, vertebrae, and wrist) were recorded. This study suggests that thiazide diuretics do not provide protection against osteoporosis. (AM J OBSTET GYNECOL 1985;152:630-4.)

Key words: Thiazides, osteoporosis, fractures, bone mineral content, postmenopausal

Osteoporosis is a condition which results from a progressive and prolonged loss of bone mineral content with advancing age. The primary mechanism for the development of osteoporosis is believed to be a gradual, increased resorption of bone over a prolonged period of time rather than a decrease in bone formation. The rate of bone resorption is increased by a number of known factors, one of which is prolonged usage of medications such as corticosteroids, anticonvulsants, aluminum-containing antacids, and thyroid supplements. Furosemide and ethacrynic acid are thought to be potentially detrimental to postmenopausal women because of their hypercalciuric activity, but thiazide diuretics have been postulated to protect against bone loss by decreasing urinary calcium excretion.

The objective of this study was to investigate the effect of thiazide diuretics on bone in postmenopausal women.

Methods

The Center for Climacteric Studies in Gainesville, Florida, has recorded the diet, medication histories, and bone mineral content of more than 1000 postmenopausal women. These women were middle-class, well-educated Caucasians, who voluntarily sought care through this research and educational center. A retrospective review of these files produced a sample of

64 women receiving thiazide medication at the time of their initial interview. Fifty-four of these postmenopausal women could be matched with postmenopausal women not receiving thiazide diuretics on the basis of the following factors: type of menopause, surgical/nonsurgical; years postmenopausal; duration of estrogen supplementation (if any), categorized as <2 years, 2-5 years, or >5 years; daily dietary and supplemental calcium and vitamin D, categorized according to <700, 700 to 1400, or >1400 mg of calcium and <400, 400 to 1000, or >1000 IU of vitamin D; and finally the Quetelet index* as an indicator of bone mass.

There was no difference >0.05 for all matched partners on the Quetelet index and no more than 5 years' difference in years postmenopausal. Women in the early menopause were matched within 1 to 2 years.

The prevalence of bone fractures was recorded for both groups based on patient history at the initial interview. Radiographs of the vertebrae were not available. Only fractures of the ribs, hips, wrists, and vertebrae were included, since these types of fractures are representative of those associated with osteoporosis. Only fractures that occurred during the duration of thiazide therapy were recorded. Likewise, fractures in the matched control subjects were recorded only if they occurred within the same time period as the duration of thiazide therapy in the matched pair.

Bone mass was recorded as the bone mineral content and bone density of the distal and midshaft of the radius for all postmenopausal women at the time of their initial visit to the Climacteric Center. The bone mineral

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*[(Weight kg)/(height cm)²] \times 100.

Table I. Matched variables for postmenopausal women receiving thiazide therapy

	•	•	•			
Matched variable	Natural menopause, on thiazide (n = 39)	Natural menopause, no thiazide (n = 39)	Surgical menopause, cn thiazide (n = 15)	Surgical menopause, no thiazide (n = 15)	Total women on thiazide (n = 54)	Total women, no thiazide (n = 54)
Age (yr)	64.5 ± 8.1*	60.3 ± 13.8	60.3 ± 12.8	53.8 ± 17.5	63.3 ± 9.7	59.8 ± 13.3
Years postmeno- pausal	16.6 ± 9.4	15.6 ± 9.3	19.3 ± 14.4	17.9 ± 13.9	17.3 ± 10.9	16.2 ± 10.7
Quetelet index (kg/cm²)	0.244 ± 0.05	0.239 ± 0.03	0.248 ± 0.05	0.246 ± 0.04	0.245 ± 0.05	0.240 ± 0.03
Duration of estrogen (yr)	0.87 ± 1.1	0.87 ± 1.1	1.73 ± 1.1	1.73 ± 1.1	1.11 ± 1.1	1.11 ± 1.1
Calcium (mg/day)	804 ± 458	896 ± 590	1152 ± 907	1046 ± 599	902 ± 627	938 ± 591
Vitamin D (IU/day)	1261 ± 4062	921 ± 3476	363 ± 381	355 ± 307	1011 ± 3469	764 ± 2958

^{*}Mean ± SD.

Table II. Diuretic formulations used by postmenopausal women (n = 54)

Diuretic	No.
Dyazide (25 mg hydrochlorothiazide, 50 mg triamterene)	30
Hydrochlorothiazide, 25 or 50 mg	14
Aldactazide (25 mg hydrochlorothiazide, 25 mg spironolactone)	2
Chlorthalidone, 50 mg	2
Apresazide (hydrochlorothiazide, 25 mg hydralazine)	1
Aldoril (25 mg hydrochlorothiazide, 250 mg methyldopa)	1
Chlorothiazide, 250 mg	1
Ser-Ap-Es (15 mg hydrochlorothiazide, 0.1 mg reserpine, 25 mg hydralazine)	1
Moduretic (50 mg, hydrochlorothiazide, 5 mg amiloride)	1
Diupres (chlorothiazide, 0.125 mg reserpine)	1

content measurements were obtained while these 54 women were receiving thiazide medications. The midshaft radius reflects mineralization of the cortical bone while the distal radius has a mixture of trabecular (25%) and cortical bone.

The Norland Digital Bone Densitometer (Model 278, Norland Corp., Ft. Atkinson, WI 53538) was used for densitometry evaluations. Depending on operator technique, the manufacturer-reported precision is in the range of 1% to 3% with an accuracy of 1% of the stated value.

The serum calcium levels, bone mineral measurements, and prevalence of fractures of the two groups were recorded after matching was completed.

An analysis of covariance was performed on the four response variables: midshaft bone mineral content, midshaft density, distal bone mineral content, and distal density. Each model tested for a difference between the diuretic and nondiuretic groups and between the surgical and nonsurgical menopause groups and for an interaction between these two factors, while controlling

Table III. Daily dosages of thiazide diuretics in postmenopausal women (n = 54)

Thiazide (mg/day)	No.	%	Average daily dose* (mg)
Hýdrochlorothiazide†			22.9 ± 11.5
100 mg	2	3.7	
75 mg	2	3.7	
50 mg	18	31.5	
25 mg	20	35.2	
<25 mg‡	8	14.8	
Unknown	1	1.8	
Chlorothiazide, 250 mg	2	3.7	
Chlorthalidone, 50 mg	2	3.7	
Total	55§	100	

^{*}Chlorothiazide, 250 mg, and chlorthalidone, 50 mg, are equivalent to 25 mg of hydrochlorothiazide diuretic activity. A total of 53 dosages of hydrochlorothiazide equivalent activity were averaged.

for the effect of a covariate. This method presumes a straight-line relationship between the response variable and the covariate (that is, a matching variable), but the slopes were allowed to differ between the two menopause groups. The χ^2 analysis was used to determine significance of fracture prevalence between the two groups. Statistical significance was established at $\alpha = 0.05$.

Results

Of the 64 postmenopausal women who received thiazide diuretics at the time of their initial bone mineral measurements, only 54 could be matched to postmenopausal women who were not taking thiazide diuretics according to the variables listed in Table I. No statistically significant differences existed between the

[†]One subject took an unknown dose of hydrochlorothiazide.

[‡]These subjects took hydrochlorothiazide as needed, every other day, or three to four times a week.

[§]One subject took hydrochlorothiazide, 25 mg, and chlorothiazide, 250 mg, each day.

Table IV. Mean bone mineral measurements in postmenopausal women taking thiazide diuretics and in matched control subjects

	Thiazid	e groub	ide group .		
Bone mineral measurements	Surgical menopause (n = 15)	Natural menopause (n = 39)	Surgical menopause (n = 15)	Natural menopause (n = 39)	p Value*
Midshaft					
Bone mineral content (gm/cm)	$0.789 \pm 0.14\dagger$	0.750 ± 0.11	0.784 ± 0.16	0.726 ± 0.13	0.48
Bone density (gm/cm²)	0.669 ± 0.09	0.620 ± 0.10	0.629 ± 0.10	0.599 ± 0.10	0.09
Distal					
Bone mineral content (gm/cm)	0.743 ± 0.21	0.753 ± 0.14	0.732 ± 0.17	0.697 ± 0.15	0.24
Bone density (gm/cm ²)	0.408 ± 0.11	0.390 ± 0.09	0.379 ± 0.10	0.384 ± 0.14	0.40

^{*}p Values are for the comparisons of combined surgical and natural menopause women in each of the two groups, thiazide and matched controls. No interaction was found between the diuretic and menopause groups in any model (p > 0.05). \dagger Mean \pm SD.

Table V. Incidence of fractures in postmenopausal women on thiazide drugs compared to that in matched controls

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Groups	No. with fractures	No. without fractures	p Value
Women on thiazides	8	42*	0.45
Women not on thiazides	12	38	

^{*}A fracture history could not be documented in four subjects on thiazide.

two groups of women as determined by these variables.

The 54 women were taking various thiazide formulations with the combination of 25 mg of hydrochlorothiazide and 50 mg of triamterene being the most frequently prescribed product (Table II). The daily dosages of the thiazide formulations are presented in Table III. Average duration of thiazide therapy was 6.1 ± 5.9 years (range of 2 to 288 months) in the 50 subjects in whom documentation could be obtained.

No significant differences were demonstrated in the response variables between diuretic and nondiuretic postmenopausal women (Table IV). However, the means of all response variables reported (midshaft bone mineral content, midshaft density, distal bone mineral content, and distal density) were higher in postmenopausal women receiving thiazide diuretics than in the control group.

There were 14 women who received thiazide diuretics during the first 5 years after the onset of menopause. Bone mineral content measurements in this group did not differ significantly from those in their matched pair.

The serum calcium levels were not significantly different between the two groups of postmenopausal women $(9.42 \pm 0.4 \text{ mg/dl})$ in the thiazide group versus $9.42 \pm 0.3 \text{ mg/dl}$ in the nonthiazide group).

The prevalence of bone fractures was not statistically different between the two groups (Table V). The thiazide group had a total of 12 fractures among eight women while the nonthiazide group had 18 fractures among 12 women (Table VI). When all types of fractures were examined among the postmenopausal women in the two groups without regard to the time limits of thiazide therapy, the matched control subjects (21/54, 38.8%) and the thiazide group (20/54, 37.0%) had similar incidences.

Comment

This study matched subjects on factors that are known to influence bone resorption in postmenopausal women, thereby allowing thiazide treatment to be independently evaluated. Therapies of at least 1400 mg of calcium per day,4 400 to 1000 IU of vitamin D per day, and estrogen replacement within 5 years of onset of menopause have been demonstrated to protect the postmenopausal woman from accelerated bone loss.5 The type of menopause a woman undergoes can also influence bone loss. Twenty-five percent of women with natural menopause lose bone at an accelerated rate, whereas up to 50% of women with surgical menopause have accelerated bone loss.6 Because obese women rarely develop osteoporosis, the Quetelet index was used to match thiazide-taking with non-thiazide-taking subjects, thereby excluding weight as a factor influencing bone resorption.

There are several factors that could affect the possible inhibitory activity of thiazide medications on bone mineral loss. Compliance with thiazide medications was unknown. The effectiveness of thiazide therapy may be significantly influenced by noncompliance or insufficient dosage. Not every factor that influences osteoporosis could be controlled, for instance, daily exercise, exposure to the sun, regularity of calcium intake, and the effect of other medications. Furthermore, the limitations of the sample size and retrospective nature of

Table VI. Number and types of fractures in postmenopausal women taking thiazides compared to matched control subjects

	7	hiazide group		Nonthiazide group						
Age (yr)	Years postruenopausal	Months on thiazide	Type of fracture*	Age (yr)	Years postmenopausal	Equivalent months†	Type of fracture			
73	23	156	V,W	81	43	276	R,R			
68	24	144	v ·	58	. 7	15	R			
64	13	24	V	72	22 .	144	H			
75	42	276	W	81	36	12	v			
56	7	108	V,V	51	6	72	W,R			
76	21	168	H	71	23	144	R,V			
63	13	24	V	75	37	276	V,R,H			
75	25	168	V,V	50	25	288 .	H,V			
				59	13	156	v			
				73	29	24	R			
				61	10	96	V			
				64	23 .	168	V			

^{*}H = Hip; R = rib; V = vertebrae; W = wrist.

the study must be considered. The bone measurements used in this study were one-time recordings after menopause and the initiation of thiazide medication. However, the group is representative of a large proportion of postmenopausal women, and the matched pair design should have controlled many of the major factors related to osteoporosis.

It has been well established that thiazide diuretics decrease urinary calcium excretion.7-9 However, differences in total serum calcium levels were not evident in this study. Mohamadi et al. 10 evaluated 11 hypertensive patients receiving 50 mg of hydrochlorothiazide twice daily for a 3-month period. The mean serum total calcium concentration was significantly higher in the thiazide-treated group than in the control subjects. However, the mean ionized calcium concentration was not significantly different between the two groups. Stote et al.11 found that subjects receiving 50 mg of hydrochlorothiazide twice daily for 25 days did have a significant increase in both total and ionized plasma calcium. These levels remained elevated for 2 weeks after treatment was discontinued. Transbol et al.12 reported no change in total serum calcium level after 37 months of 5 mg of bendroflumethiazide treatment in early postmenopausal women. Christiansen et al.13 showed an initial increase of total serum calcium in subjects on a regimen of thiazide therapy but the values returned to basal levels by 12 and 24 months of treatment. This paralleled a transient inhibition of bone resorption. The subjects tested were women in their early menopause who received 5 mg of bendroflumethiazide daily.

It has been proposed that, because the elevation of serum calcium may be a transitory effect, the inhibition of bone loss may be demonstrated only within the first several years of the onset of menopause. Within our study population, 14 women received thiazides within the first 5 years after the onset of menopause. Serum calcium levels and bone mineral content measurements did not differ from those in the matched pairs. Thus, based on this small sample, a transitory inhibitory effect of thiazides on bone was not evident. Consequently, any protective effect by thiazides during the later years of menopause would seem unlikely.

The lack of a demonstrable significant difference in bone measurements of thiazide-treated postmenopausal women has been reported by other investigators. 12. 13 A 2-year study of 315 women in early menopause (0.5 to 3.0 years after cessation of menses) was conducted to determine the inhibiting effects of bendroflumethiazide (5 mg daily) on bone resorption.12 Bone mineral content was measured every 3 months in both distal forearms by photon absorptiometry. Thiazides had only a transient inhibition on bone resorption which was apparent for the first 6 months of the trial. After 2 years, the thiazide and control groups showed decreases in bone mineral content of 1.5% and 3.3%, respectively. In a second trial by the same investigators, the bone mineral content in 63 women in early menopause who received bendroflumethiazide (5 mg daily) was measured over a 3-year period.13 As in the first study, bone mineral content remained constant during the first 6 months of therapy. However, at the end of the 3-year trial, there was no significant difference in bone mineral content between the postmenopausal women receiving thiazide diuretics and those receiving the placebo. Bone mineral content averaged 94.1% of baseline in the control group and 95.2% in the thiazidetreated group (p > 0.50). The authors stated that the initial beneficial effect on bone mineral loss might be the result of a decreased bone turnover (less bone re-

[†]Equivalent months to the duration of thiazide therapy in the matched pair.

sorption than bone formation), until a new steady state of bone metabolism was achieved. These investigators concluded that the long-term prevention of postmenopausal bone loss by thiazide diuretics is doubtful.¹³

In contrast to the ineffectiveness of thiazides to inhibit postmenopausal bone loss, a recent retrospective study of 323 elderly Japanese men (mean age 68) reported bone resorption to be significantly influenced by thiazide therapy.³ Men receiving a minimum daily dose of 25 mg of hydrochlorothiazide for a mean duration of 7.25 years were compared to 1045 untreated, normotensive men. Bone mineral content measured by photon absorptiometry at five skeletal sites was significantly increased in the thiazide-treated sample.

Estrogen in the woman and testosterone in the man function to maintain skeletal integrity. However, in women the precipitate reduction in estrogen levels is an acute event occurring at the onset of menopause while in men the decline in testosterone is a more subtle and gradual regression. During the first 5 years of menopause women have been reported to lose bone mineral content at a rate six times that of men.1 Not until a woman has reached her 60s will this rate of loss decline and become similar to that of her male counterpart. The difference between thiazide effect on bone mass in elderly men and postmenopausal women may be explained by the abruptness of menopause. Thus in the early postmenopausal woman (6 months to 5 years) the abrupt loss of estrogen and the accelerated rate of bone loss may eventually override a transient, beneficial effect of thiazide therapy. In the elderly man, whose loss of bone mass is more gradual, thiazide therapy may help protect against progressive bone loss.

The lack of demonstrable differences in bone mineral measurements was further confirmed by a similar prevalence of bone fractures in the two groups. The intended therapeutic outcome of thiazide therapy in postmenopausal women would be a decreased risk of bone fractures. The data from this retrospective study do not indicate that thiazide therapy would be beneficial in this regard.

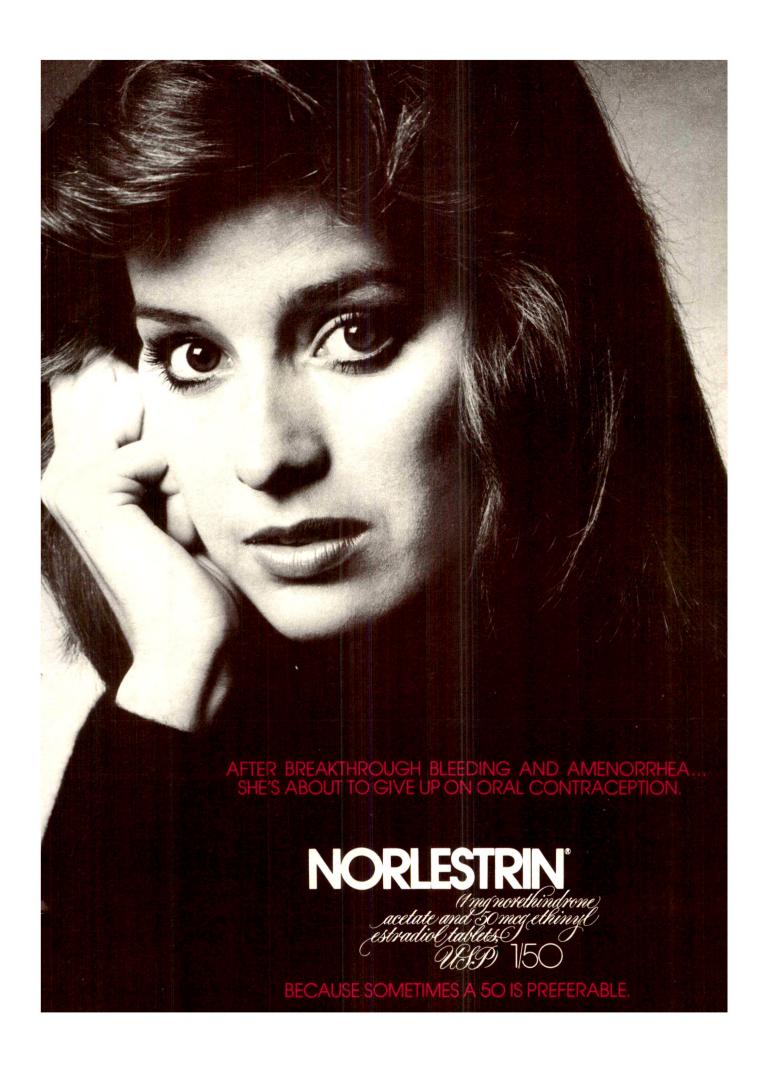
One study demonstrated a reduction in the incidence of bone fractures in elderly women taking a thiazide diuretic. ¹⁴ Women on a regimen of thiazide therapy had a fracture incidence of 6.6% compared to an incidence of 11.3% in women not taking any antihypertensive

medication. However, information on duration of thiazide treatment, years postmenopausal, types of fractures, time of fractures, and the use of estrogen, calcium, and vitamin D were not available.

The results of this retrospective evaluation of thiazide therapy as an inhibitor of bone mineral loss showed no significant differences between the women who were taking thiazide medication and those who were not receiving thiazide diuretics. This is further confirmed by a similar prevalence of bone fractures in the two groups.

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Brief Summary of Prescribing Information
NORLESTRIN* (norethindrone acetate and ethinyl estradiol tablets, USP)

ee section under Special Notes on Administration and HOW SUPPLIED.

Before prescribing, please see full prescribing information **DESCRIPTION**

Nortestrin Products are progestogen-estrogen combinations INDICATIONS AND USAGE

INDICATIONS AND USAGE.

Norlestrin Products are indicated for the prevention of pregnancy in women who elect to use oral contraceptives as a method of contraception.

In clinical trials with Norlestrin 1/50 involving 25,983 therapy cycles, there was a pregnancy rate of 0.05 per 100 woman-years; in clinical trials with Norlestrin 2.5/50 involving 96,388 cycles, there was a pregnancy rate of 0.22 per 100 woman-years.

Dose-Related Risk of Thromboembolism from Oral Contraceptives: Studies have

Lose-neighbor hisk of informoembolism from Ural Contraceptives: Studies have shown a positive association between the dose of estrogens in oral contraceptives and the risk of thromboembolism. It is prudent and in keeping with good principles of therapeutics to minimize exposure to estrogen. The oral contraceptive prescribed for any given patient should be that product which contains the least amount of estrogen that is compatible with an acceptable pregnancy rate and patient acceptance.

- an acceptable pregnancy rate and patient acceptance
 CONTRAINDICATIONS

 1. Thrombophlebitis or thromboembolic disorders
 2. A past history of deep-ven thrombophlebitis or thromboembolic disorders
 3. Cerebral vascular or coronary artery disease
 4. Known or suspected carcinoma of the breast

 - 4. Known or suspected carcinoma of the breast
 5. Known or suspected estrogen-dependent neoplas-a
 6. Undiagnosed abnormal genital bleeding
 7. Known or suspected pregnancy (See WARNING No. 5)
 8. Benign or malignant liver tumor which developed during the use of oral contraceptives or other estrogen-containing products

 (ADMINIOS.)

or other estrogen-containing products

WARNINGS

Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. The risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke. The use of oral contraceptives should be strongly advised not to smoke. The use of oral contraceptives should be trongly advised not to smoke. The use of oral contraceptives should be familiar with the following information relating to these risks.

1 Thromboembolic Disorders and Other Vascular Problems. An increased risk of thromboembolic and thrombolic disease associated with the use of oral contraceptives is wellgestablished. Studies have demonstrated an increased risk of fatal and nonfatal venous strong causes, it was estimated that the risk of hemorrhagic and thrombotic.

Cerebrovascular Disorders: In a collaborative study in women with and without predisposing causes, it was estimated that the risk of hemorrhagic stroke was 2.0 times greater in users than nonusers, and the risk of thrombotic stroke was 4.0 to 9.5 times greater. Myocardial Infarction: An increased risk of myocardial infarction associated with cral contraceptives has been reported confirming a previously suspected association. These studies found that the greater the number of underlying risk factors (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of preeclamplic foxermal) for cornary artery disease, the higher the risk of developing myocardial infarction regardless of whether the patient was an oral contraceptive user or not. Oral contraceptives, however were found to be a clear additional risk factor.

It has been estimated that users who do not smoke (smoking is considered a major predisposing condition to myocardial infarction) are about twice as likely to have a statal myocardial infarction as nonusers who do not smoke for a contraceptive user o

have about a fivefold increased risk of fatal infarction compared to users who do not smoke, but about a tenfold to twelvefold increased risk compared to nonusers who do not smoke. The amount of smoking is also an important factor.

Risk of Dose: In an analysis of data. British investigators concluded that the risk of thrombosis, is directly related to the dose of estrogen used in oral contraceptives, however the quantity of estrogen may not be the sole factor involved.

Persistence of Risk: Two studies have suggested that an increased risk may persist for a long as 6 years after discontinuation of oral contraceptive use for cerebrovascular disease and 9 years for myocardial infarction. In addition, a prospective study suggested the persistence of risk for subgraphing themorphage. for subarachnoid hemorrhage

sistence or risk for subaraching hemornage. Estimate of Excess Mortality from Circulatory Diseases: The risk of diseases of the cir-culatory system is concentrated in older women, in those with a long duration of use, and in

culatory system is concentrated in older women, in those with allong duration of use, and in cigarette smokers.

A study of available data from a variety of sources concluded that the mortality associated with all methods of birth control is low and below that associated with childbirth, with the exception of oral contraceptives in women over 40 who smoke. The risk of thromboembolic and thrombotic diseases associated with oral contraceptives increases with age after approximately age 30 and, for myocardial infarction, is further increased by hypertension, hypercholesterolema, obesity diabetes, or history of pre-ectamptic toxemia, and especially by cigarette smoking. The physician and the patient should be alert to the earliest manifestations of thromboembolic and thrombotic disorders. Should any occur or be suspected, the drug should be discontinued immediately.

A fourfold to sixtloid increased risk of postsurgery thromboembolic complications has been reported in users, at feasible, oral contraceptives should be discontinued at least four weeks before surgery of a type associated with an increased risk of thromboembolism of prolonged immobilization.

prolonged immobilization 2. Ocular Lesions, such as optic neuritis or retinal thrombosis have been associated with the use of oral contraceptives. Discontinue the oral contraceptive if there is unexplained sudden or gradual, partial, or complete loss of vision, coset of proptosis or diplopia; papilledema, or retinal vascular fesions.

3. Carcinoma. Long-term continuous administration of estrogen in certain animal species increases the frequency of carcinoma of the breast, cervix, vagina, and liver. In humans, an increased risk of endometrial carcinoma associated with the prolonged use of exogenous estrogen in postmenopausal women has been reported. However, there is no evidence suggesting increased risk of endometrial cancer in users of conventional combination or progestogen-only oral contraceptives.

Studies found no evidence of increase in breast cancer in women taking oral contraceptives however an excess risk in users with documented bening hereast disease was

tives; however, an excess risk in users with documented benign breast disease was

tives, however, an excess risk in users with documented benigh breast disease was reported. There is no confirmed evidence of an increased risk of cancer associated with draf contra-ceptives. Close clinical surveillance of users is, nevertheless, essential. In cases of undrag-nosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be taken to rule out malignancy. Women with a strong family history of breast cancer or who have breast nodules, fibriocystic disease, or abnormal mammograms, should be monitored with patient as care.

or who have breast nodules, fibrocystic disease or abnormal mammograms should be monitored with particular care.

4. Hepatic Tumors. Benign hepatic adenomas have been found to be associated with oral contraceptives. Because hepatic adenomas may rupture and may cause death through intra-abdominal hemorrhage, they should be considered in women presenting abdominal pain and tenderness, abdominal mass, or shock.

A few cases of hepatocellular carcinoma have been reported in women taking oral contraceptives. The relationship of these drugs to this type of malignancy is not known at this time. 5. Usage in or Immediately Preceding Pregnancy, Birth Defects in Offspring, and Malignancy in Female Offspring. During early pregnancy, female sex hormones may seriously damage the offspring.

An increased risk of congenital anomalies, including heart defects and limb defects, has been reported with the use of oral contraceptives in pregnancy.

been reported with the use of oral contraceptives in pregnancy.

There is some evidence that triploidy and possible other types of polyploidy are increased. among abortuses from women who become pregnant soon after ceasing oral

contraceptives.

Pregnancy should be ruled out before continuing an oral contraceptive in any patient who has missed two consecutive menstrual periods. If the patient has not adhered to the sched-

ule, the possibility of pregnancy should be considered at the time of the first missed period

ule, the possibility of pregnancy should be considered at the time of the first missed period and oral contraceptives should be withheld until pregnancy has been ruled out. It pregnancy is confirmed, the patient should be apprised of the potential risks to the fetus and the advisability of continuation of the pregnancy should be discussed.

Women who discontinue oral contraceptives with the intent of becoming pregnant should use an alternate form of contraception for a period of time before attempting to conceive. Administration of progestogen-only or progestogen-estrogen combinations to induce withdrawal bleeding should not be used as a test of pregnancy.

6 Gallbladder Disease. Studies report an increased risk of surgically confirmed gallblad der disease in users of oral contraceptives.

7 Carbohydrate and Lipid Metabolic Effects. Because decreased glucose tolerance has been observed in a significant percentage of patients, prediabetic and diabetic patients should be carefully observed while receiving oral contraceptives.

An increase in triglycerides and total phospholipids has been observed.

8 Elevated Blood Pressure. An increase in blood pressure has been reported in patients receiving oral contraceptives. The prevalence in users increases with longer exposure. Ag is also strongly correlated with development of hypertension. Women who previously have had hypertension during pregnancy may be more likely to develop elevation of blood pressure.

pressure

9. Headache. Onset or exacerbation of migraine or development of headache of a new
pattern which is recurrent, persistent, or severe, requires discontinuation of oral

pattern which is recurrent, persistent, or severe, requires uracontraceptives

10. Bleeding Irregularities. Breakthrough bleeding, spotting, and amenorrhea are frequent reasons for patients discontinuing oral contraceptives. In breakthrough bleeding, nonfunctional causes should be borne in mind. In undiagnosed abnormal bleeding from this vagina, adequate diagnostic measures are indicated to rule out pregnancy or malignancy. Women with a past history of oligomenoirhea or secondary amenorrhea, or young wome without regular cycles should be advised that they may have a tendency to remain anovulatory or to become amenorrheic after discontinuation of oral contraceptives.

11. Ectopic Pregnancy Ectopic as well as intrauterine pregnancy may occur in contracetive failures.

12 Breast-Feeding. Oral contraceptives may interfere with lactation. Furthermore, a small fraction of the hormonal agents in oral contraceptives has been identified in the milk of molt. ese drugs

- ers receiving these drugs.

 PRECAUTIONS

 1. A complete medical and family history should be taken prior to the initiation of oral contraceptives. The prefreatment and periodic physical examinations should include special reference to blood pressure. breasts, abdomen, and pelvic organs, including Papanicolao smear and relevant laboratory tests. As a general rule, oral contraceptives should not be prescribed for longer hann one year without another examination.

 2. Preexisting uterine leiomyomata may increase in size.

 3. Patients with a history of psychic depression should be carefully observed and the drudiscontinued if depression recurs to a serious degree.

 4. Oral contraceptives may cause fluid retention and should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated.

 5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence of jaundice. If jaundice develops, the medication should be discontinued.

 6. Steroid hormones may be poorly metabolized and should be administered with caution in patients with impaired liver function.

 7. Users may have disturbances in normal tryptophan metabolism, which may result in a relative pyridoxine deficiency.

 8. Serum folate levels may be depressed.

 9. The pathologist should be advised of oral contraceptive therapy when relevant specimens are submitted.

 10. Certain endocrine and liver function tests and blood components may be affected.

ens are subremed. 10. Certain endocrine and liver function tests and blood components may be affected.

Drug Interactions: Reduced efficacy and increased incidence of breakthrough bleeding have been associated with concomitant use of ritampin. A similar association has been suggested with barbiturates, phenylbutazone, phenyloin sodium, fetracycline, and ampicillin ADVERSE REACTIONS.

An increased risk of the following serious adverse reactions has been associated with oral contraceptives, thrombophiebitis, pulmonary embolism, coronary thrombosis, cerebral hrombosis, cerebral hrombosis, cerebral hemorrhage; hypertension, gallbladder disease; benign hepatomas,

contraceptives thrombophiebitis; pulmonary embolism, coronary thrombosis, cerebral thrombosis, cerebral hemorrhage; hypertension, gallbladder disease; benign hepatomas; corigential anomalies.

There is evidence of an association between the following conditions and the use of oral contraceptives, although additional confirmatory studies are needed, mesenteric thrombosis, neuro-ocular lesions, eg., retinal thrombosis and optic neuritis. The following adverse reactions have been reported in patients receiving oral contraceptives and are believed to be drug related, nausea and/or vomiting, usually the most common adverse reactions, occur in approximately 10% or less of patients during the first cycle. Other reactions as a general rule, are seen much less frequently or only occasionally gastrontestinal symptoms, breakthrough bleeding, spotting, change in menstrual flow dysmenorrhea almencrihea during and after freatment, temporary intertility after discontinuance of treatment, edema, chloasma or melasma, breast changes; change in weight, change in cervical ecrivical secretion, possible diminition in lactation when given immediately postpartum, cholestatic jaundice, migraine, increase in size of uterine leiomyociata, (ash (allergic); mental depression, reduced tolerance to carbohydrates.

The following adverse reactions have been reported and the association has been neithe confirmed nor refuted, premenstrust-like syndrome, characts, changes in libido; chorea, changes in appetite, cystitis-like syndrome, headache, nervousness, dizziness; hirsuitim, loss of scalap har erythema multiforme, erythema nodosum, hemorrhagic eruption, vagintis porphyria.

porpryria

Special Notes on Administration

Menstruation usually begins two or three days, but may begin as late as the fourth or tifth
day, after discontinuing medication

After several months on treatment, bleeding may be reduced to a point of virtual absence

ay be a result of medication and not indicative of pregnancy

HOW SUPPLIED

reduced flow may be a result of medication and not indicative of pregnancy.

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High failure rates in outpatient treatment of salpingitis with either tetracycline alone or penicillin/ampicillin combination

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Atlanta and Savannah, Georgia, Seattle, Washington, Baltimore, Maryland, San Francisco, California, and Brooklyn, New York

Eight hundred twenty-five ambulatory women with a clinical diagnosis of salpingitis were randomized to a 10-day course of either penicillin/ampicillin or tetracycline. Forty-four percent of women had gonococcal salpingitis and 56% nongonococcal salpingitis. Overall, both regimens cured equal proportions of women: At 30 days, 81% were cured by penicillin/ampicillin and 82% by tetracycline. However, the proportion of women with gonococcal salpingitis cured by 30 days was significantly greater than that of women with nongonococcal salpingitis. By 30 days, 14% of women with gonococcal salpingitis and 21% of women with nongonococcal salpingitis were not cured by either regimen. These data suggest that both regimens were only marginally acceptable for women with gonococcal salpingitis and that neither regimen was acceptable for nongonococcal salpingitis. (AM J OBSTET GYNECOL 1985;152:635-41.)

Key words: Salpingitis, gonococcal and nongonococcal, penicillin/ampicillin, tetracycline

Salpingitis is an acute and chronic infectious disease of women of immense public health importance because it is a major cause of infertility, ectopic pregnancy, loss of productivity, and human suffering. A recent international symposium underscored the worldwide extent of this problem but did not address the issues of appropriate antimicrobial therapy at any length.¹

In 1974, the United States Public Health Service published its first guidelines for the management of salpingitis.² Ten days of penicillin with ampicillin or tetracycline alone were the suggested regimens for treating ambulatory women. When these recommendations were revised in 1979,³ these two schedules were again the recommendations of choice, although they had never been thoroughly tested in field trials.

In the 1982 Sexually Transmitted Diseases Guidelines,⁴ salpingitis was given a treatment category of its own. Recommended therapies now include cefoxitin, which is active against penicillinase-producing gonococci, certain aerobic gram-negative rods, and many anaerobes; and combined therapy with a variety of βlactam antibiotics and tetracyclines. However, many physicians treating salpingitis in outpatients will continue to rely on a penicillin and a tetracycline, either alone or in combination because of the difficulty of obtaining adequate specimens from the fallopian tubes and the lack of availability of many laboratories for processing specimens for chlamydia or anaerobes; if endocervical cultures are taken at all, they are likely to be processed only for *Neisseria gonorrhoeae*. The choices for antibiotic therapy are also limited; cefoxitin must be administered by injection, and it is unlikely that single-dose therapy with any antibiotic will be sufficient to eradicate nongonococcal infections. Also, both cefoxitin and doxycycline, one of the currently recommended tetracycline compounds, are expensive, thus placing them outside consideration for use by many public clinics.

Our study compared the efficacy of 10 days of a penicillin/ampicillin combination with that of 10 days of tetracycline alone for the treatment of gonococcal and nongonococcal salpingitis in ambulatory women. Our data suggest that both regimens were only marginally acceptable for women with gonococcal salpingitis and that neither schedule was acceptable for nongonococcal salpingitis.

Methods

Patient population. Patients were enrolled between March, 1976, and December, 1978, at the Boston City Hospital; The Johns Hopkins Medical School, Baltimore; the Memorial Medical Center, Savannah; the Southwestern Medical School, Dallas; the San Francisco City Hospital; and the University of Washington School of Medicine, Seattle.

Study eligibility. Women could enter the study if they had lower abdominal pain for 14 days or less, did

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Table I. Therapy, type of salpingitis, and racial distribution by study area

The second secon									10%		100	 (1) (1) (2) (1) (1) (2) (2) (3) 	2012/01/01/01	
	7	otal	В	oston	Bal	timore	Sav	annah	De	allas	1	San incisco	Se	attle
	N_{θ} .	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Total	832		62		107		132		195	, , , , , , , , , , , , , , , , , , , ,	78		258	
Treatment	825	100.0	62	100.0	106	100.0	132	100.0	189	100.0	78	100.0	258	100.0
APPG/	405	49.1	28	45.2	54	50.9	66	50.0	93	49.2	38	48.7	126	48.8
Amp.*														
Tet.†	420	50.9	34	54.8	52	49.1	66	50.0	96	50.8	40	51.3	132	51.2
GC‡	822	100.0	61	100.0	107	100.0	131	-100.0	191	100.0	74	100.0	258	100.0
Positive	358	43.6	17	27.9	60	56.1	55	42.0	99	51.8	22	29.7	105	40.7
Negative	464	56.5	44	72.1	47	43.9	76	58.0	92	48.2	52	70.3	153	59.3
Race	826	100.0	62	100.0	107	100.0	132	-100.0	195	100.0	78	100.0	252	100.0
Black	548	66.3	53	85.5	100	93.5	117	88.6	155	79.5	42	53.9	81	32.1
White/	278	33.7	9	14.5	7	6.5	15	11.4	40	20.5	36	46.2	171	67.9
other														

^{*}APPG/Amp. = Aqueous procaine penicillin G, 4.8 mU, plus 1 gm probenecid plus ampicillin, 2.0 gm/day for 10 days.

not require hospitalization, and fulfilled certain criteria for the diagnosis of salpingitis (see next section). Women with recurrent episodes of salpingitis were also eligible for the study. We excluded women for any of these reasons: pregnancy, antimicrobial therapy within 2 weeks of the admission visit, or allergy to any of the study drugs.

Criteria for diagnosis. A diagnosis of salpingitis was established by history and a bimanual pelvic examination positive for at least three of the following: direct abdominal tenderness to palpation, tenderness on manipulation of the uterine cervix, adnexal tenderness or masses, or lower abdominal rebound tenderness. These were the clinical criteria which, when found together, have been most highly predictive of laparoscopically confirmed salpingitis.⁵

The on-site investigator calculated an index of disease activity for each patient, termed the severity score, which provided a semiquantitation of the extent of disease and permitted assessment of both changes in the course of the disease during follow-up examinations and comparisons between study sites. Eight indicators of pelvic inflammation, direct and rebound abdominal tenderness, decreased bowel sounds, cervical motion tenderness, and right and left adnexal tenderness and enlargement, were given numerical values from 0 to 3 indicating increasing degrees of severity. The individual values were totaled to provide a single numerical index of severity of disease ranging from 0 (no disease) to 24. This score has been useful in predicting antibiotic treatment failure in women hospitalized with salpingitis.6

Criteria for hospitalization. Women were hospitalized if the diagnosis of salpingitis was in question, if a large pelvic mass was palpated, if the illness was severe,

or if the patient was vomiting. None of these patients was enrolled in our study.

Enrollment procedures. At the first visit, the investigator obtained written informed consent, a standardized history, and an endocervical specimen for N. gonorrhoeae. Women with positive cultures for N. gonorrhoeae were classified as having gonococcal salpingitis, and those with negative cultures as having nongonococcal salpingitis. All patients were randomly assigned to one of the two treatment regimens, according to a random numbers list generated at the Centers for Disease Control. Patients received either aqueous procaine penicillin G, 4.8 million units intramuscularly, together with a gram of probenecid orally, followed by ampicillin monohydrate, 0.5 gm four times a day orally for 10 days; or tetracycline hydrochloride, 1.5 gm orally as a single loading dose, followed by tetracycline, 0.5 gm four times a day orally for 10 days. The same lots of antibiotics were used in all locations.

Determination of therapy-produced cure. Investigators decided if the patient was cured at each follow-up visit. The decision was based on the severity scores and predetermined criteria: (1) the clinician's judgment on the necessity of switching to another antibiotic regimen during the 10 days of initial therapy with a protocol drug, (2) the need to continue another antibiotic regimen after the 10 days of initial therapy, or (3) the need to reinstitute therapy with any antibiotic schedule, either standard or nonprotocol, any time after completion of the initial regimen except if the patient was considered cured at any previous follow-up visit and there was a minimal interval of 2 weeks before return with new signs and symptoms of salpingitis; this was considered to be a new episode.

Microbiologic techniques. All specimens were plated

[†]Tet. = Tetracycline hydrochloride, 2.0 gm/day for 10 days.

[‡]Endocervical gonococcal infection.

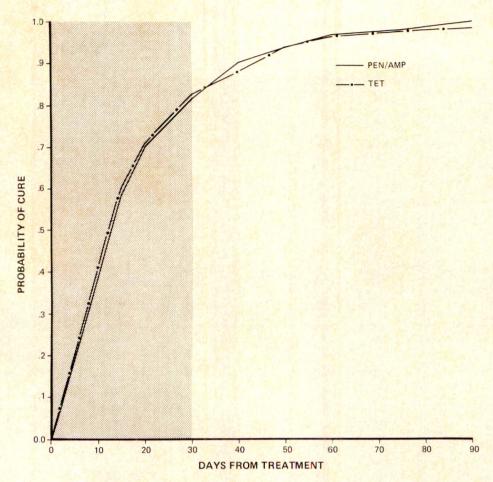


Fig. 1. Pelvic inflammatory disease cure curves with penicillin/ampicillin and tetracycline.

immediately onto Thayer-Martin medium, brought to the laboratory in a candle-extinction jar within 2 hours, and incubated at 35° C in a 5% carbon dioxide atmosphere. Colonies were harvested and frozen at -70° C in trypticase soy broth and were shipped to Atlanta on dry ice. Isolates were confirmed as N. gonorrhoeae by sugar utilization tests (Minitek), and antimicrobial susceptibility patterns to penicillin and tetracycline were determined according to an agar dilution method.7

Statistical analysis. Survival (lifetime) analysis of data was used as the primary method. This technique is used when interest is in the time to occurrence of some event, often time to death or to occurrence of disease, particularly when the period of study is long enough that the probability of occurrence of the outcome event cannot be considered constant. Then the overall probability should be calculated as a function of the probabilities of several intervals. In this study, the outcome variable is cure and the time taken to produce the cure.

We grouped the data into time intervals rather than

considering individual times to cure. When estimating the probability of cure within each interval, we used the actuarial method of analysis; that is, in estimating the probability that a woman is cured in the i-th interval, given that she is infected beyond the (i-1)th interval, we assumed that any withdrawal occurred midway in the interval.

Differences in the overall curves were tested with the Mantel-Haenszel (Mantel-Cox) test.8 This test is useful when there are consistent differences between two study variables, for example, testing whether the cure probabilities are greater for treatment 1 than for treatment 2 within each of the intervals. We also used Breslow's version of the Wilcoxon test,9 which gives more weight to the earlier intervals.

Results

Eight hundred thirty-two women were recruited at the six medical centers over 2 years. Four hundred five were randomized to the 10-day penicillin/ampicillin regimen and 420 to the 10-day course of tetracycline. The remaining seven were treated with nonstudy an638 Thompson et al.

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Table II. Mean value of selected characteristics of patients by area

	Total			- 124	Boston			Baltimore		
	No.	Mean	SE	No.	Mean	SE	No.	Mean	SE	
Age (yr)	830	22.3	0.2	62	24.3	0.7	107	22.5	0.6	
Severity score	832	9.7	0.1	62	10.0	0.4	107	11.0	0.3	
Days antibiotic taken	668	9.9	0.1	50	9.8	0.5	91	9.5	0.2	
Temperature (degrees Fahrenheit)	794	99.5	0.1	58	98.8	0.1	104	99.8	0.2	
Weight (pounds)	721	134.9	1.1	47	134.3	3.8	100	137.9	2.9	
Previous PID* (No. of episodes)	780	0.63	0.0	55	0.73	0.2	103	0.6	0.1	
Previous gonorrhea (No. of episodes)	793	0.71	0.0	62	0.53	0.1	104	0.7	0.1	

^{*}PID = Pelvic inflammatory disease.

Table III. Selected characteristics of study patients and study exclusions

	Study	group	Excl	usions	D 1 1 1 0	
	No.	%	No.	%	Excluded % of total	Total
Total	736		96	- 1-11	11.5	832
Area						002
Boston	62	8.4	0	0.0	0.0	62
Baltimore	94	12.8	13	13.5	12.1	107
Savannah	115	15.6	17	17.7	12.9	132
Dallas	168	22.8	27	28.1	13.8	195
San Francisco	64	8.7	14	14.6	17.9	78
Seattle	233	31.7	25	26.0	9.7	258
Treatment						
APPG/Amp.*	359	49.0	46	50.0	11.4	405
Tet.*	374	51.0	46	50.0	11.0	420
GC*					The state of the s	
Positive	317	43.3	41	45.6	11.5	358
Negative	415	56.7	49	54.4	10.6	464
Race						
Black	495	67.8	53	55.2	9.7	548
White/other	235	32.2	43	44.8	15.5	278
Initial severity score (mean ± SE)	9.7	± 0.1	9.7	± 0.4	9.7 ± 0	
Age (yr) (mean ± SE)	22.4	± 0.2	21.5	± 0.4	22.3 ± 0	.2

^{*}For explanations see footnotes to Table I.

tibiotic combinations and were not included in the analysis of antibiotic efficacy. After randomization, no differences existed between the two treatment groups for the following characteristics: type of salpingitis, race, age, admission severity score or temperature, mean days of antibiotic therapy, or weight. Of the 825 patients for whom racial data were available, 548 (66.3%) were black and 276 (33.7%) were white or of other races (Table I). There were 358 women with gonococcal salpingitis (43.5%) and 464 with nongonococcal salpingitis.

No significant differences in the means of any characteristic occurred among study areas (Table II). Ages ranged from 10 to 50 years, with a mean of 22.5 years; 58% of the total, or 81% of the 604 patients for whom this information was available, had incomes below \$5000 a year. Thirty-seven and one-half percent had

had at least one other episode of salpingitis (range of one to eight episodes). Of women with nongonococcal salpingitis, 39.4% had previous episodes of infection compared with 35.0% of women with gonococcal salpingitis. Thus 60.6% of women with nongonococcal salpingitis were apparently experiencing their first clinical episode. Racial composition and type of salpingitis were the two most variable characteristics among study areas.

Ninety-six women (12%) were not seen again after the initial visit and were removed from analysis of cure. No major differences were found between patients who dropped out and those returning for at least one visit, except that white women were lost to follow-up more often than black women (Table III).

Overall, the proportions cured on the tetracycline and penicillin/ampicillin regimens were the same when

	Savannah			Dallas Sæn Francisco		Dallas Sæn Francisco					Seattle	
No.	Mean	SE	No.	Mean	SE	No.	Mean	SE	No.	Mean	SE	
132	22.1	0.4	195	20.9	0.3	78	23.6	0.6	256	22.5	0.3	
132	9.8	0.3	195	10.7	0.2	78	9.2	0.4	258	8.5	0.2	
99	10.6	0.3	152	9.6	0.1	62	10	0.2	214	9.8	0.2	
128	99.3	0.1	178	100.6	0.1	77	99.0	0.1	249	99.1	0.1	
123	135.1	2.8	157	132.9	2.3	78	131.9	3.3	216	136.2	1.8	
107	0.6	0.1	183	0.6	0.1	76	0.6	0.1	256	0.7	0.1	
117	0.7	0.2	179	0.6	0.1	75	0.6	0.1	256	0.9	0.1	

etiologic classification was not taken into account (Fig. 1). The overall median time to cure, that is, the time at which half of the patients were cured, was 13 ± 1.4 days for those who received penicillin/ampicillin and 12 ± 1.4 days for those who received tetracycline. At 30 days 81% were cured by penicillin/ampicillin and 82% were cured by tetracycline irrespective of type of salpingitis (Table IV). Thus, while the proportions cured at 30 days with the two antibiotic regimens in each area were not significantly different, the median time to cure in Baltimore was different for penicillin/ ampicillin and tetracycline. There were also no overall differences in the cure curves between gonococcal and nongonococcal salpingitis (Fig. 2). However, the proportion of women with gonococcal salpingitis cured at 30 days (0.86) was significantly different from that of women with nongonococcal salpingitis (0.79). Seattle, with the largest number of participants, accounted for most of this difference. There, 81.7% of women with gonococcal salpingitis, compared to 64.5% with nongonococcal salpingitis, were cured by 30 days (p = 0.004).

We pooled data to provide a summary estimate of the efficacy of the two antibiotic regimens for gonococcal and nongonococcal salpingitis (Tables V and VI). We could detect no differences in the overall cure curves for the two antibiotics. The median days to cure for each antibiotic for either gonococcal or nongonococcal salpingitis were not significantly different (Table V). However, there is a suggestion that within the penicillin/ampicillin treatment group, women with gonococcal salpingitis had a greater chance of being cured within 30 days than women with nongonococcal salpingitis (0.05 < p < 0.1). An important point to be made is that 14% of women with gonococcal salpingitis and approximately 21% of women with nongonococcal salpingitis had not been cured by 30 days.

Noncompliance with the 10-day antibiotic regimen could affect cure rates. We considered women to be noncompliant if they took antibiotics for less than 10 days and did not have side effects. Noncompliant

Table IV. Percent of women with pelvic inflammatory disease cured by 30 days of follow-up by treatment within area

Area	Treatment	Proportion cured	SE
Boston	APPG/Amp.*	69.3	9.9
	Tet.*	82.8	8.5
Baltimore	APPG/Amp.	74.5	7.1
	Tet.	90.5	5.4
Savannah	APPG/Amp.	96.0	2.7
	Tet.	89.3	4.5
Dallas	APPG/Amp.	88.1	4.2
	Tet.	91.9	3.0
San Francisco	APPG/Amp.	88.7	6.7
	Tet.	83.7	7.7
Seattle	APPG/Amp.	73.0	4.3
	Tet.	70.0	4.4

^{*}For explanations see footnotes to Table I.

women were less likely to be cured at 30 days than those who completed the full 10-day course (p=0.009). However, there were only 29 individuals in this group, that is, only 2.6% of women taking ampicillin and 3.3% of women taking tetracycline. All were noncompliant because of side effects, either rash or gastrointestinal upset. No serious reactions occurred in any patient.

Of 221 isolates of *N. gonorrhoeae* isolated before treatment (no isolates were available from Dallas), 6% were resistant to $\geq 0.500~\mu g/ml$ of penicillin and 31.2% to $\geq 0.500~\mu g/ml$ of tetracycline. Thirty-eight strains were recovered from women at follow-up visits after therapy was completed; 5% were resistant to $\geq 0.500~\mu g/ml$ of penicillin and 3% were resistant to $\geq 0.500~\mu g/ml$ of tetracycline.

Comment

Overall, we could detect no difference in the end results of cure for the penicillin/ampicillin and tetracycline regimens. Baltimore was the only site at which there was a difference between the two regimens. There, women treated with tetracycline responded more rapidly than women treated with penicillin/am-

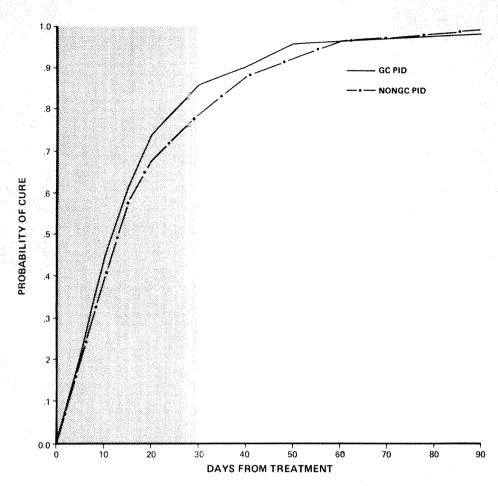


Fig. 2. Pelvic inflammatory disease cure curves for both gonococcal and nongonococcal types.

Table V. Median days to cure for gonococcal and nongonococcal pelvic inflammatory disease: Results by treatment

				, a 5 1x
Treatment drug	Culture result	No. in sample	Median days to cure	Difference in survival curves
APPG/ampicillin*	GC†	163	11.8	p = 0.28
	Non-GC	193	13.8	p – 0.26
Tetracycline	GC	151	1,1.7	0.00
	Non-GC	222	12.5	p = 0.20

^{*}APPG = Aqueous procaine penicillin G plus probenecid.

Table VI. Percent of women with gonococcal and nongonococcal pelvic inflammatory disease cured at 30 days by antibiotic therapy

Culture Treatment drug result	Cured (%)	SE
APPG/ampicillin* GC†	85.2	2.9
Non-GC	77.4	3.4
Tetracycline GC	86.4	3.1
Non-GC	79.7	2.9

^{*}APPG = Aqueous procaine penicillin G plus probenecid. †Endocervical gonorrhea.

picillin; Baltimore also had the highest incidence of gonococcal salpingitis. We were not able to demonstrate a correlation between the proportion cured at any area and the antibiotic sensitivity patterns of the gonococci to penicillin or tetracycline in that area. There were also no overall differences in cure curves between gonococcal and nongonococcal salpingitis, although women with gonococcal salpingitis fared better at 30 days than women with nongonococcal infections. This observation was statistically significant only in Seattle. However, the most important point is that by 30 days

[†]Endocervical gonorrhea.

14% of women with gonococcal salpingitis and 21% of women with nongonococcal salpingitis treated with either antibiotic schedule were not cured.

Several difficulties in performing and interpreting data from antibiotic therapy studies of salpingitis may have affected our results. First, the case definition of salpingitis on clinical grounds alone is suspect. Recently investigators have developed a series of major and minor criteria for diagnosis. 10 The clinical criteria we used in this study are similar to those proposed above. Second, it is difficult to determine when a patient is actually cured. We attempted to avoid this difficulty by using the severity score and multiple examinations by the same clinician. We were not able to completely resolve the problem of interobserver variability in grading cases even though we had general agreement by examiners on how to score the examination. In addition, we do not know the normal range at the low end of the scale in particular, nor do we know the "presalpingitis" score for any of our patients. Clinician differences may have contributed to some of the differences in the proportion cured that we observed between sites. Finally, we lack knowledge about the natural history of salpingitis when untreated. It is clear that in some patients, perhaps most, the acute episode will resolve spontaneously with time and the disease will enter a chronic or quiescent state, but the actual dynamics of that are not known. Therefore it is sometimes difficult to decide, particularly in clinically mild cases, whether antibiotics were important in recovery.

Because patients entered and left the study continuously, we felt that life-table techniques were the most appropriate method of analysis and that the curves derived from the analysis were the easiest way to display time-based data. This technique also allowed visual comparisons between the various study groups. It is important to remember that these curves represent smoothed out aggregates of all the study sites. The curve shapes by site are actually quite variable. We also felt that it was reasonable to look at cure at a single point in time. We chose 30 days because the experience of the investigators and most clinicians suggested that most patients were well on the way to recovery by this time. Also, this was the time when decisions were often made, after one or two courses of antibiotics, to hospitalize the patient for an elective pelvic surgical procedure if there were persistent or residual adnexal masses.

Like other sexually transmitted diseases, in the urban setting, salpingitis is a disease of the young and underprivileged. The mean age of our patients was 22 years, and one third had already had one or more episodes before the episode that brought them to this study. In the outpatient setting, nongonococcal salpin-

gitis was more common than gonococcal disease. Only in Dallas and Baltimore were there more gonococcal than nongonococcal cases, and even in those cities, the proportion was only slightly higher than 50%.

Antibiotics that did not cure gonorrhea 15% of the time or chlamydial cervicitis 20% of the time within a month would be considered unsatisfactory, and alternative treatments would be developed which would provide a cure at least 95% of the time. We submit that the same reasoning should apply to salpingitis. We do not believe that either of the regimens we studied should be accepted as optimal treatment at this time. Other antibiotic options, including longer duration of therapy, should be studied in controlled trials. In addition, the effect of intrauterine contraceptive device removal at the time of treatment should be included in the treatment plan. Our data suggest that the most severely ill women responded more slowly to treatment, so perhaps a study should be designed to include hospitalization as part of the treatment plan. Ultimately it may be shown that these results are the best that can be expected with any antibiotic combination we can devise and that they may be the most cost effective if hospitalization costs are included. At the very least, women treated with these antibiotics as outpatients need close monitoring not only during the first month of treatment to identify those that clearly need alternative or extended treatment but during the long term to identify that group of women who might benefit from repeated screening for sexually transmissible agents and repeated courses of antibiotics.

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The response of the New York Obstetrical Society to the report by the New York Academy of Medicine on maternal mortality, 1933-1934

Robert F. Porges, M.D.

New York, New York

My topic for this address concerns an event in the life of the New York Obstetrical Society. During the past year, I went to the rare books section of the library of the New York Academy of Medicine, the repository of the archives of this Society. At the beginning I had no clear idea of what my subject for this address was going to be. Apart from the illustrious careers and important contributions of the many fellows, which are well known to most of you, the accomplishments of this society in its separate life are more obscure. Although monthly meetings have been held with faithful regularity here at the Yale Club since 1927, there seemed to be no record of any special linkage between the society and the important obstetric events of the time, with one notable exception. From Claude Heaton's History of the First One Hundred Years of the Society through 1963, my attention was directed toward and came to rest on a thick dossier which held information about the dramatic events of the season of 1933 to 1934, exactly 50 years ago, and they took place here, perhaps in this room.

The president of our society for the 1933-1934 season was Dr. Edward Bullard. The membership consisted of 69 active fellows, 35 life fellows, and 10 non-resident fellows. The first three programs of that year, which included case presentations and a study of the time of ovulation by Dr. Kurzrok, gave no indication of the explosion that was to rock the foundation of the society with the announcement that appeared on the opening page of *The New York Times* on Monday, November 20, 1933 (Fig. 1). As you can see, the top headlines concerned local and national events of no enormous consequence, but farther down on the page (Fig. 2) was an article of consuming interest about the brew-

ers, a situation which had come to a head at exactly that time, and paired with it was an article on childbirth deaths.

Together with publication by the Commonwealth Fund of New York of a report entitled, "Maternal Mortality in New York City, a Study of All Puerperal Deaths 1930-1932," the Medical Information Bureau of the New York Academy of Medicine released to the press an abstract of the book, under the title "Why Women Die in Childbirth." Interestingly, the abstract survives intact, although the whereabouts of the original report, of more than 290 pages, has proved to be elusive.

Background

"In the year 1933, the maternal mortality rate in the United States was 61.9 per 10,000 live births. In the year 1950, it was 8.7 per 10,000 live births. Prior to 1930, the rate had not diminished but had rather shown a rising trend: 60.8 in 1915, 64.7 in 1925, and 67.3 in 1930. The persistence of this high maternal mortality rate in the earlier years of the century, in contrast to the progressive fall in the general mortality rate which was already well under way, stimulated the interest of obstetricians in trying to find reasons for this discrepancy. Among those so interested and concerned was Dr. George W. Kosmak, who as a member of the Public Health Relations Committee of the New York Academy of Medicine was familiar with the overall picture, and who as a teacher and practitioner of obstetrics realized that something must be wanting in the teaching and practice of his specialty. In 1917, he first suggested to the committee that it undertake a study of the subject. This it agreed to do and through a subcommittee distributed a questionnaire to all hospitals that rendered obstetric service in New York City. The data obtained were found to be inadequate for the formulation of any definite conclusions, and no report was issued. Ten years later, another attempt was made by a study of the figures of the Bureau of Vital Statistics, but again without results. Nothing daunted, Dr. Kosmak persisted in his efforts, so that in 1928, the Public Health Relations Committee deputed him, along with Ralph W. Lobenstine, to submit plans for a study of the phases of

From the Department of Obstetrics and Gynecology, New York University School of Medicine.

Presidential address, presented at the meeting of the New York Obstetrical Society, May 15, 1984.

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Reprint requests: Dr. Robert F. Porges, Department of Obstewics and Gynecology, New York University School of Medicine, 550 First Ave., New York, NY 10016.

The New York Times.

TWO CE NEW YORK, MONDAY, NOVEMBER 20, 1933. ROOSEVELT URGES LAGUARDIA SEEKS Roosevelt Is Asked to Intervene To Protect Scottsboro Negroes **BIG FEDERAL FUND WELLES TO REMAIN** farning of 'Massacre' of Seven Prisoners and Their Lawvers of AS ENVOY TO CUBA FOR CITY PROJECTS Deratur (Ala.) Court Today, Defense Counsel Wire President a Plea to Obtain State Troops. Plans Aid for Sewage Plant. Ambassador Agrees at a Long By F. BAYMOND DANIELL. Conference With Presiden Incinerators, Subway and New Public Buildings. at Warm Springs. GRAU ASKED HIS RECALL

Fig. 1. The New York Times, page 1, headlines, November 20, 1933.

the public health problems of obstetrics as they affected New York City. These plans were accepted and their implementation was entrusted to an obstetric advisory subcommittee that consisted of Dr. Kosmak, Dr. John Polak, Dr. Aronow, and Dr. Benjamin P. Watson, with Dr. Ransom S. Hooker as director of the study.

"The New York Obstetrical Society granted a loan for the initial expenses, and the Commonwealth Fund financed the work as it went along and also the final publication."*

In the 3 years embraced by the study, from 1930 through 1932, 2041 maternal deaths occurred in New York City. Of this number, the committee estimated that 1343 (65.8%) could have been prevented "if the care of the woman had been proper in all respects." The committee devoted its major attention to the preventable deaths and, by a careful review of every death that occurred, established, to the extent possible, the factor or factors responsible for the fatality. The Registrar's office of the Department of Health agreed to furnish each week a photostatic copy of every death certificate which stated or implied a puerperal condition as the cause of the death. Each case reported was investigated through a field staff of doctors and nurses. This investigation included an interview with the doctor in attendance and a survey of the hospital record, the hospital facilities, and the home conditions. As the tabulation of these facts was completed, each case was studied and discussed by the committee without the members having knowledge of the doctor or the hospital concerned.

Responsibility for the occurrence of the 1343 deaths which the Committee adjudged to have been prevent-

*From Watson BP. History of the New York Academy of Medicine Committee on Maternal Mortality. Am J OBSTET GYNECOL 1954;68:12-4.

able was distributed among physicians, patients, and midwives. To the medical group, 61.1% of the preventable deaths were charged; the patient was held to be responsible for 36.7%, and the midwife for 2.2%, of the total number of these deaths.

Among the factors included in the report were the widespread use of anesthesia, the decline in spontaneous deliveries, the greater frequency with which operative measures were used, cesarean sections, hospital and home deliveries, prenatal care, and economic status. An elaboration of some of the more important points in this report include the following. I quote.

"The use of anesthesia during labor and delivery has grown steadily in extent since its introduction in the last century, and is a problem of the most pressing importance, more so in the USA than in any other country. This has come about largely through pressure from the lay public. The women of the large urban centers have become steadily more insistent in their demands for shorter less painful parturition, and the accoucheur may disregard these demands only at great risk to his practice.

"The frequent use of instrumentation is based upon the easy accessibility of anesthesia. In comparing the maternal deaths in operative deliveries and those in which the delivery was spontaneous, the committee found that the maternal mortality was five times as high among operative deliveries as among the spontaneous ones. The death rate for spontaneous deliveries is less than one fifth that for the operative. Clearly, this represents a serious defect in the management of these cases. It is not contended that the rates can be made equal: the necessity for operative interference arises, at times, out of serious abnormalities or disturbances of the mechanisms of labor, which, in themselves, greatly increase the hazards.

BREWERS ASK CURB CHILDBIRTH DEATHS ON STRONGER BEER HELD 65% NEEDLESS

Would Popularize 3.2 by Cut- Medical ting Tax to \$3 a Barrel, Blames With \$7 for Higher Brew. Such

Medical Academy Report Blames Doctors for 61% of Such Mortality Here.

AS AID TO TEMPERANCE TOO MANY OPERATIONS

Return of Five-Cent Glass Is Overuse of Anesthesia Also
Aim of Proposal Made by Charged — Fewer Die in
Them at Washington. Homes Than in Hospitals.

Fig. 2. The New York Times, page 1. Lower section of page.

"While all surgical maneuvers have increased in obstetrics, the incidence of cesarean sections has increased notably. In 1910, in one of the large New York hospitals, only two in 1000 deliveries were made by cesarean section. In 1927, the number increased to 25.

"A little less than 30% of all deliveries studied during the 3-year period took place in the home. The relative death rate per thousand live births for hospital and home deliveries are 4.5/1000 in the hospital and 1.9/1000 in the home. It should be remembered that only those deliveries which are unassociated with serious abnormalities are usually undertaken in the home. In reviewing the matter, the committee observes that 'the great increase in hospitalization for the normal parturient patient has failed to bring the hoped-for reduction in puerperal morbidity and mortality."

New York City at that time had 863 licensed midwives, who attended approximately 10% of annual births. The committee interviewed 59 midwives and adjudged 19 of them to be competent; 20 were thought to be "only fairly competent," and 20 were incompetent. When judged on the basis of the results, the record of midwife deliveries compared favorably with that of physician deliveries. The committee found that "there is no great disparity between the results of the work done by the two groups." Contrary to the generally accepted opinion, the committee stated that "the midwife is an acceptable attendant for properly selected cases of labor and delivery."

The committee made several concrete suggestions, as shown in the article in *The New York Times*: "There is a great need to educate the lay public as well

as the medical profession to understand the necessity for change in certain of the methods now employed. Sixty percent of all deaths, according to the Committee, which could have been avoided, were brought about by some incapacity in the attendant: lack of judgment, lack of skill, or careless inattention to the details of the case. Some of these situations have arisen out of the fact that interns have been given too wide a field of independent activity. Most are plainly the result of incompetence. Prevention in this field will mean increasing the respect of the physician for the gravity of obstetric operations and educating him to greater caution in attacking problems which are properly the field only of the highly trained obstetrician. The importance of prenatal care must be emphasized. The medical profession is obligated to inform the lay public that operative delivery undertaken merely to alleviate pain or shorten labor involves increased risk for both mother and baby. The relative safety of home delivery should be emphasized. Hospitals must have qualified obstetricians as directors of staff. Hospital facilities must be dedicated to the obstetrical patient, with separate wards, isolation facilities, and separate delivery rooms, with masking. The less experienced members of the staff must be under the supervision of responsible heads. The situation with regard to midwives must be altered. More schools are needed to train them; licensing should be based upon examination, the physician must be prepared to give the midwife unqualified cooperation" [paraphrased from The New York Times].

According to the editorial in *The New York Times*, "The hazards of childbirth in New York City are greater than

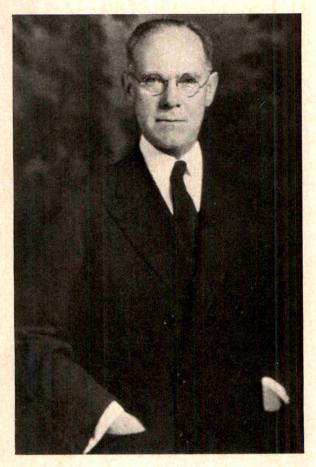


Fig. 3. Photograph of Dr. Ransom S. Hooker. (Kindly provided by Mrs. Dyson Duncan.)

they need be. Responsibility for reducing them rests with the medical profession."

The principals

I was surprised at first not to find the name of the director of the study, Dr. Ransom S. Hooker (Fig. 3), listed among the fellows of this Society. Investigation, however, disclosed that Dr. Hooker was a general surgeon, in fact, a former Director of Surgery at Bellevue Hospital, First Division, from 1921 to 1925. He attended Columbia University College of Physicians and Surgeons, class of 1900, and trained at Roosevelt and St. Luke's Hospitals. During World War I, Dr. Hooker served overseas as a major in the Army Medical Corps. Later, he organized a 3000-bed hospital in Le Mans, France. He was decorated by the French government. According to his obituary in The New York Times, he took a special interest in exploration, and only illness in his family prevented his accompanying Admiral Robert Peary's North Pole expedition in 1909. One of his ancestors, Lyman Hall, of Georgia, signed the Declaration of Independence. In a recent telephone conversation, Mrs. Dyson Duncan, of Mount Kisco, New York, Dr. Hooker's daughter, explained that her father

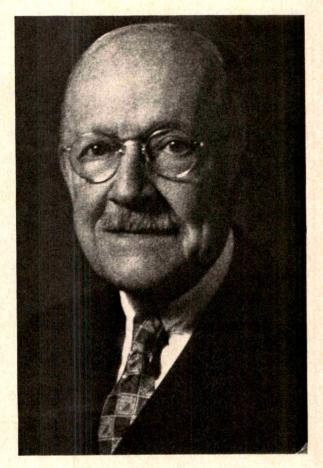


Fig. 4. Photograph of Dr. George W. Kosmak.

had died on a train en route to New York from his home in Charleston, South Carolina. This apparently led to some legal and administrative difficulties, but all were brushed aside when it was learned that her father had been on the staff at Bellevue Hospital. Mrs. Duncan explained further that at the time of the report she was expecting her first child, much to the great concern of her father, whose impression of obstetric practices had been severely prejudiced. Subsequent to publication of the report, Dr. Hooker apparently took no further part and retired from active practice several years later. The most visible catalyst for the development of this report was Dr. George W. Kosmak (Fig. 4), a graduate of Columbia University College of Physicians and Surgeons, class of 1899. The following biographical material was gleaned from the memorial resolution presented to this Society by Dr. Howard C. Taylor, Jr., in 1954.

"His interest and energies extended far beyond either private or hospital practice of obstetrics and gynecology. He was president of this Society in 1920 and again in 1930. In 1909, he became the editor of the American Journal of Obstetrics. In 1920, he founded the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY and edited it until 1952.

"Dr. Kosmak will be remembered for his fearless attack upon the problem of Maternal Mortality. In this endeavor he had his greatest opportunity in 1930, when he served as a member of the committee of the New York Academy of Medicine which made its now famous analysis of the causes of puerperal morbidity, the publication of which resulted in a controversy which raged nowhere more fiercely than within this society. These disagreements are now nearly forgotten, but there persists the statistical method of self-criticism fostered in no small part by that committee and which has done so much to improve American obstetrics.

"Dr. Kosmak's peculiar position was that he served in no small way as the collective intelligence of his specialty. He was supremely interested in its organization and devoted his efforts to the national scene rather than to intramural politics. He was a critic and philosopher of the practice of obstetrics" [paraphrased from Dr. Taylor's resolution].

In a recent telephone conversation with Dr. Taylor, who, as you know, succeeded Dr. Kosmak as editor of the Gray Journal, Dr. Taylor claimed to have few specific recollections of those events.

Dr. Benjamin P. Watson was professor and chairman of obstetrics and gynecology at Columbia University College of Physicians and Surgeons, Dr. Taylor's immediate predecessor, and a member of the original committee. He is recalled by Dr. Taylor as a very good surgeon.

Response

As might be expected, this report and the manner of its presentation aroused a storm of professional protest. At a meeting of the Council of the New York Obstetrical Society, on November 29, 1933, the president and secretary were directed to file a formal protest to the New York Academy of Medicine against the publicity given to the report by the Information Bureau of the Academy.

President Bullard sent a letter to Dr. Sachs, of the New York Academy of Medicine, insisting that the study was originally undertaken for the information of the medical profession. In their response, the Academy referred to a consistent spirit of cooperation between Dr. Ransom Hooker, Dr. Iago Galdston, the executive secretary of the Medical Information Bureau, and Mr. Graham Taylor, of the Commonwealth Fund.

Furthermore, according to the academy's subcommittee, "... most of the unfavorable criticism which followed the publicity in the lay press arose from the startling nature of the facts disclosed by the report itself. Had there been any attempt to minimize the seriousness of the situation as presented in the report and given to the lay press, it would undoubtedly and justly have resulted in an unfavorable reaction, with a

charge that the profession was shielding itself against attack."

Dr. Iago Galdston, the former executive secretary of the Medical Information Bureau, is now 88 years old and divides his time between his home in Connecticut and his psychiatric practice in Brooklyn Heights, New York. In a recent phone conversation, Dr. Galdston took credit for interpreting the original report to the press. In fact, he later authored a book on maternal mortality, in 1937, and he referred to the event as a triumph of communication. "After all," he said, "look how the maternal mortality declined after this report, before antibiotics or effective blood transfusions."

Today, officials at the Commonwealth Fund are unable to retrieve the relevant file, thanks to vigorous purging of its old records by zealous newcomers. However, according to the News-Letter of the Commonwealth Fund, January, 1934, ". . . this study was greeted by the most extensive publicity ever accorded to any Fund publication. It led 310 newspapers in 39 states to devote 2,355 column inches to the report. Within the publishing trade it was analyzed as a test of intelligent newshandling of this almost 100% news item." Despite this success, I believe that the report by Dr. George Papanicolaou on exfoliative cytology, also published by the Commonwealth Fund, in 1941, probably had a greater impact on medicine. In any event, the Commonwealth Fund managers were making interesting choices.

At the regular meeting of the Society in January, 1934, it was moved, seconded, and carried that the February meeting of the Society be a symposium devoted to the academy's report. Dr. Bullard gave a résumé of the action taken by the Council in regard to the recent newspaper article. Dr. Kosmak moved that the president appoint a committee to cooperate with the New York Academy of Medicine for the purpose of arranging a combined meeting to discuss the report, with a view to a constructive program. This motion was seconded and passed. All of these events were not devoid of certain elements of humor. The page in the Health Examiner announcing the report by the Academy was faced apparently by a full-page advertisement for a popular contraceptive. The advertising copy bordered on the lurid, and Dr. Bullard protested that "...the advertisement occupied a conspicuous position with reference to the review of the Maternal Mortality Report and would lead to the thought that this was a prearrangement." There are also several voluminous letters in the file from a physician to Dr. Bullard complaining that the span of the time covered by the report specifically favored the hospitals of one of the committee members.

At a regular meeting of the Society on February 13, 1934, a symposium was held to discuss the report of

the New York Academy of Medicine. Sixty-nine members and 33 guests were in attendance. The discussions that followed the formal presentations were perhaps even more illuminating as a window on some of the authoritative obstetric opinions of the day.

Dr. Alfred C. Beck, of Brooklyn, New York, professor of obstetrics at the Long Island College of Medicine, and author of a noted textbook of obstetrics, pointed out that "...it would have been more acceptable to have reduced the deaths due to abortion and ectopic pregnancy, resulting in a mortality ratio of one death in 222 live births." Concerning the relative merits of operative and spontaneous deliveries, of physician and midwife care, and hospital and home confinements, "I do not see," said Dr. Beck, "how the report contains sufficient data on these important questions to warrant conclusion. Likewise, any analysis which fails to consider the greater risk that is entailed during the first labor also is incomplete."

Dr. Samuel Cosgrove, from the Margaret Hague, stated that, "In spite of my intense sympathy with the woman who is already engaged in the work of midwifery, I most strenuously desire to express my own opposition to an increase in the number of midwives and an increase in the relegation of obstetrics to midwife practice. We in our state believe that the midwife is with us largely by reason of persistence of old-world standards among a certain proportion of our population. Just before the war, the midwife delivered 30% of all maternity cases in our state. Today, she delivers around 10%. We think that she is an anachronism, that she is being necessarily and progressively eliminated by economic stress, and we do not want to do anything to perpetuate her being; but as long as she is with us, a necessary but not particularly desirable condition, we are going to help her do her work as best she can. It is of course admitted that operative obstetrics is abused in all the ways the Committee describes. The orgy of unrestrained gynecic surgery is but a generation past ... better balanced operative obstetrics is surely on the way."

Dr. George H. Ryder commented that, "The recommendations of the Committee are good so far as they go, but they are inadequate. Birth control is not mentioned... Widespread instruction in birth control would reduce maternal mortality. Every man in this room knows it to be so. To rely on midwives is to lean on a broken reed. No real advance can be expected of them. It seems strange that the medical profession should be willing to delegate such an important part of its work to those outside the profession."

Dr. Onslow A. Gordon, Jr., asked, "Has the best possible skill, both in diagnosis and treatment been used? If this patient had had the advantage of the best possible attention, would this death have occurred? When

these superlative criteria have been used by a small committee of experts, it naturally leads to an enormous number of so-called preventable deaths. What would a like consideration of all the medical and surgical deaths in New York City reveal as regards the question of preventability? An attempt should have been made to classify operative deliveries as low forceps and episiotomy as opposed to high forceps and manual dilation." Dr. Gordon was also critical of the safety of home deliveries. On the matter of education, he said, "We do not need more hours for obstetric training in the medical school curriculum, but fewer hours spent demonstrating such things as the technique of version, the introduction of extraovular and intraovular bags, and the technique of craniotomy. All of these should be none of the affairs of a medical student."

Dr. Harvey B. Matthews remarked that, "With intensive study, one realizes the extent and scope of the work and becomes convinced that the Committee really did its best to be 'fair' and 'square' in its decisions. I am for the report—almost 100%. It will do more to promote better obstetrics than anything that has happened to obstetrics in the last 75 years, or since Oliver Wendell Holmes wrote his famous essay on 'Contageousness of Childbed Fever.' The Bible says, 'Spare them, Oh, God! who confess their faults.' Let us confess our faults and get together and do something about it."

In summarizing, George Kosmak had the following to say. "I would like just a few moments in defense because I think a defense is needed of the Committee itself. The Committee expected from the beginning that it would get a few kicks, and the proceedings this evening have certainly made us realize that expectation. I think that the four of us who constituted that committee may have some difficulty in sitting down for the next few days. We advocated the midwife in a certain field because she is already in that field, and we must do something about it. She has to be taken care of. I just wish that everybody here could have sat in on those monthly meetings which the committee had, to discuss the facts that were brought out in this report. I think, gentlemen, that you would have been ashamed of yourselves as members of a profession that would allow such things to go on as those that were brought to our attention. Whether we liked it or not, we had to admit that they were shortcomings of medical men, of our own colleagues, and there is nobody who can correct that situation except the members of the profession themselves. It was appalling to think that any man, a graduate of medicine and perhaps a graduate of a hospital, would do some of the things that we heard about. For example, a man does a version, tears the body away from the head, and the head is left in utero. Then he promptly does a cesarean section, with the assistance of his brother, and gets the head out. That is not an

isolated instance, but an example of many. Therefore, you must excuse the committee for being prejudiced because of what they heard about these things. Remember that the committee, in formulating its recommendations, had to bear in mind that it was not dealing with the 70 obstetric specialists who are members of this Society, but that it was dealing with a membership of 4000 doctors in this city, and we could not base our standards on what the 70 specialists in obstetrics would do. We needed to base our conceptions of what should be done on what was an average for these 4000 practitioners. Gentlemen, the important thing in the correction of this situation is to get next to the general practitioner, because he still does most of the obstetric procedures. Another thing is that we thought that not all the fault could be laid at the door of the general practitioner who did not know what to do. We believed that a great many of the cases were due to the interns and junior members of the staff who were allowed to do major obstetric procedures without consulting their seniors. That finding cropped up repeatedly. In some very large and well-known institutions, the junior members of the staff were allowed at night to do major obstetric procedures for which they were not fitted, either by training or anything else. This society cannot afford to sit by and let this thing go unchallenged."

On March 7, 1934, a meeting was held at the New York Academy of Medicine, under the joint auspices of the Academy, the New York Obstetrical Society, and the Medical Society of the County of New York, to discuss the constructive aspects of the Academy's report. Dr. Kosmak summarized his presentation by recommending (1) more effective education and training, undergraduate and postgraduate, of doctors and nurses; (2) encouragement of home deliveries under proper auspices; (3) development of a certified list of practitioners competent and willing to take confinement cases in the home; (4) creation of an Obstetric Advisory Council to the Commissioner of Health, which shall develop standards for obstetric practice as applied to hospitals and practitioners, and regularly inspect obstetric facilities in hospitals of every type, and (5) a survey and better control of midwife practices.

The Report of the Committee of the New York Obstetrical Society to Review the Maternal Mortality Report of the Public Health Relations Committee of the New York Academy of Medicine was presented at the regular meeting of the Society in April, 1934. This report was prepared by a committee that consisted of Harvey Matthews, George Ryder, Henricus Stander, and Edwin Langrok, and was chaired by Eliot Bishop. Several interesting points were raised which was to be the society's official response.

"The society believes that the valuable and excellent

report of the Academy Committee has, in its publication in the lay press, been misrepresented.... The society believes it is necessary to issue this statement both to correct false impressions previously created, and to give its authoritative opinion on controversial subjects connected with childbirth in New York.... All obstetricians know that operative deliveries at times are absolutely necessary for the safety of the baby or mother, and that with good indications and in skilled hands they are merciful, life-saving, and constitute one of the greatest advances in modern obstetrics. The society deplores the performance of cesarean section as done too frequently with insufficient indication by the unfit. But the fact remains that the operation is absolutely essential to life of mother and baby in many instances due to the physical structure of the mother, or for other causes.'

The society's committee thought that it was impossible in all cases to determine preventability and believed that the report had gone too far in placing this responsibility. Instead of judging a death to be preventable, it seemed wiser to judge such a death as being associated with a controllable cause. The committee believed that the release to the newspapers should have stated that the high maternal death rate in New York City was due to controllable causes, since the conclusions in regard to preventability and responsibility might result in unfair criticisms or even unjust lawsuits for malpractice. The committee declared against any increase in the number of midwives. The recommendation was that the inadequate hospital be placed under the supervision of a regional committee, in cooperation with the department of hospitals. The committee reaffirmed the proper and humane use of anesthesia and analgesia. The medical schools in New York City should allow as much time for the undergraduate teaching of obstetrics as was allotted to surgery and medicine. The academy report did not emphasize that 40% of deaths occurred in early pregnancy and from nonpuerperal causes, with 477 being due to abortions and ectopic pregnancies and 344 to extrapuerperal causes. Consequently, only 1220 of the 2041 deaths listed occurred in actual childbirth from puerperal causes. This gave a maternal mortality rate in actual childbirth from puerperal causes of 3.5 per 1000.

Perhaps most important was the Committee's declaration that, "in this vicinity, the New York Obstetrical Society is the most authoritative body in any matter pertaining to childbirth, and as such this Society should go on record in its own archives, as well as in the lay press, as to its opinion on all controversies, including cesarean sections, anesthesia, home and hospital deliveries, the complications of pregnancy, labor and the puerperium."

In conclusion, a review of the evidence makes it abun-

dantly clear that the membership was polarized and hard feelings did linger. Dr. Kosmak and Dr. Watson resigned from the council, and Dr. Watson, who was in line to become the next president, did not in fact become president of the society until 1937. According to Dr. Heaton's history, the report of the New York Obstetrical Society eventually was published in the American Journal of Obstetrics and Gynecology. I could find no evidence for that beyond two specific letters of rejection by Dr. Kosmak, and a third by Franklin Martin, editor of Surgery, Gynecology and Obstetrics. I believe that it was printed privately and never distributed very widely, which was regrettable because, in my view, it was an excellent report, and far more representative of the advanced obstetric beliefs of that day.

The result of all these meetings, reports, and turmoil was the creation of local maternity mortality review committees and a further reduction in maternal mortality. The persistence to this day of an Obstetrical Advisory Committee to the Commissioner of Health is further evidence of the constructive recommendation of the report of the New York Obstetrical Society. All of the aforementioned events are chronicled in *The New York Obstetrical Society—An Historical Sketch—1863-1963*,

by Claude Edwin Heaton (F. A. Davis Company, 1963), in Obstetrics & Gynecology in America: A History, by Harold Speert, M.D. (Waverly Press, Inc., for the American College of Obstetricians and Gynecologists), and by Benjamin Watson in a paper published in 1954 in the American Journal of Obstetrics and Gynecology on the occasion of a Festschrift for George W. Kosmak; and the rest is, as they say, spread over the minutes of the society's meetings. Few of the members of that era are with us today. Beyond the ones whom I have mentioned, no active efforts were made to reach some of the others.

Apart from their historical interest and the neat coincidence that all of these events occurred exactly 50 years ago, are there any lessons for the future? Outgoing presidents of this society recently have spoken in defense of elitism and excellence. It seems to me that the willingness of this society to respond so responsibly in the past may serve as an appropriate model for us in the present and future assumption of community leadership in professional affairs, which leadership it has been suggested in some quarters we must again adopt.

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Asymptomatic parturient women with high-virulence bacteria in the amniotic fluid

Ronald S. Gibbs, M.D., Jorge D. Blanco, M.D., K. Lipscomb, R.N., and P. J. St. Clair San Antonio, Texas

This study describes the postpartum course of asymptomatic parturient women who had $\geq 10^2$ cfu of high-virulence (HV) bacteria per milliliter of amniotic fluid. Of 60 asymptomatic parturient women with $\geq 10^2$ cfu of HV bacteria per milliliter of amniotic fluid, 27 (48%) remained asymptomatic in the puerperium, 16 (27%) developed fever only, and 17 (28%) developed endometritis. In asymptomatic versus symptomatic women, there were no statistically significant differences in number or type of isolates or in length of membrane rupture or labor-to-collection interval. However, there were significant differences in the intervals from collection to delivery and in the rate of cesarean section delivery. For comparison, 40 of these patients were matched with women in whom only low-virulence organisms were detected in the amniotic fluid. In the HV group, 16 women (40%) remained asymptomatic, 15 (37.5%) developed fever only, and nine (22.5%) had endometritis. In the low-virulence group, 27 women (67.5%) remained asymptomatic, 10 (25%) developed fever only, one (2.5%) developed endometritis 10 days post partum, and two (5%) had other infections (p < 0.01). Clinically evident uterine infection depends upon type and numbers of bacteria in utero, duration of bacteria in utero, and route of delivery. (AM J OBSTET GYNECOL 1985;152:650-4.)

Key words: High-virulence bacteria, amniotic fluid, asymptomatic, postpartum fever, endometritis

During labor, women with high-virulence bacteria in the amniotic fluid often have intra-amniotic infection.¹ Others, who have cesarean section delivery, develop endometritis after cesarean section,² but some remain asymptomatic.¹ The purpose of this report is to describe the postpartum course of asymptomatic parturient women who had ≥10² cfu of high-virulence bacteria per milliliter of amniotic fluid. For comparison, we describe the puerperal course of matched women in whom only low-virulence organisms were detected in the amniotic fluid.

Methods

This study was performed at Medical Center Hospital (formerly Bexar County Hospital), San Antonio, Texas. The obstetric patients were indigent and predominantly Mexican-American. After informed consent had been obtained, samples of amniotic fluid were collected from patients who were at high risk for infection. In general, these patients had had ruptured membranes for more than 10 to 12 hours before collection of the sample of amniotic fluid. The sample was

collected by aspiration of a transcervical, intrauterine catheter, after the first 7 ml was discarded. None of the patients had fever or other specific signs of intra-amniotic infection, or had received antibiotics in labor.

After collection, the specimens were transported to the laboratory in a capped plastic syringe. Within approximately 30 to 60 minutes after collection, the specimen was quantitatively inoculated on a variety of media for isolation of aerobes and anaerobes. Qualitative cultures were performed for genital mycoplasmas.³

In view of recognized differences in pathogenicity among the many microbial species isolated in genital infections in previous studies, we attempted to distinguish high- from low-virulence organisms. However, not all authorities would agree on the categorization of isolates, such as enterococci, Streptococcus viridans, Gardnerella vaginalis, and Mycoplasma hominis. Accordingly, in this analysis, we included in the high-virulence organisms only the following: Staphylococcus aureus, group A or B streptococci, aerobic Gram-negative rods, Neisseria gonorrhoeae, anaerobic Gram-positive cocci, Clostridium sp., Bacteroides sp., and Fusobacterium sp. Patients were considered to be acceptable in the high-virulence group if their amniotic fluid contained one or more of these isolates (either with or without low-virulence isolates).

Clinical courses were categorized as follows: asymptomatic—no genital tract symptoms and all temperatures ≤99.4° F; fever only—transient, early-onset temperature ≥99.8° F, but no localizing signs or symptoms

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Table I. Characteristics of amniotic fluid cultures from 60 asymptomatic parturient women with high-virulence bacteria in the specimen

	Puerperal course							
Characteristic	Asymptomatic (n = 27)	P^I	Fever only (n = 16)	P^2	Uterine infection $(n = 17)$	P^3		
Isolates, mean ± SD	2.3 ± 0.9	NS	2.8 ± 1.3	NS	2.7 ± 1.3	NS		
High-virulence bacteria only	30%	NS	19%	NS	29%	NS		
Aerobes and anaerobes	67%	NS	75%	NS	64%	NS		
≥10° cfu/ml	37%	NS	57%	NS	47%	NS		
≥10 ⁵ cfu of high- virulence bacteria/ml	26%	NS	38%	NS	37%	NS		
M. hominis	21% (4/19)	NS	42% (5/12)	NS	17% (2/12)	NS		
U. urealyticum	53% (10/19)	NS	42% (5/12)	NS	50% (7/12)	NS		
Leukocytes on stain	52%	NS	75%	< 0.02	35%	NS		

- P1, Compares asymptomatic and fever-only groups.
- P2, Compares fever-only and uterine infection groups.
- P⁸, Compares asymptomatic and uterine infection groups.

and without use of either prophylactic or therapeutic antibiotics; endomyometritis—fever ≥100° F and other signs of uterine infection, with use of therapeutic antibiotics.

To control for duration of rupture of membranes and mode of delivery, we attempted to match each patient in the high-virulence group on these two characteristics with a patient whose amniotic fluid had lowvirulence organisms only. Organisms considered to be of low virulence were lactobacilli, Staphylococcus epidermidis, diphtheroids, Propionibacterium sp., and Eubacterium lentum. Patients were included in the low-virulence group if Ureaplasma urealyticum was found in the amniotic fluid, but excluded if M. hominis was present.3 Because some samples of amniotic fluid were collected many hours prior to delivery, and because patients with such samples may have acquired high-virulence bacteria subsequent to collection of the amniotic fluid, we arbitrarily excluded from the low-virulence group those with delivery more than 4 hours after collection of the sample. Thus, we will report descriptive data on the total group of patients with high-virulence bacteria and comparative data on the matched pairs.

Clinical data on maternal and neonatal courses were collected prospectively. Each patient was evaluated for late puerperal complications (within 6 weeks) by telephone interview or review of inpatient and outpatient charts. Statistical analysis was performed by χ^2 analysis or by Fisher's Exact Test, wherever appropriate, for discrete data and by paired t test for continuous data.

Results

During the period of June, 1979, to January, 1984, 60 asymptomatic patients fulfilled the criteria for this study. These women accounted for approximately 7% of those sampled.

Descriptive data. The largest group (n = 27; 48%) remained asymptomatic. Sixteen (27%) developed fever only, and 17 (28%) developed endometritis. An analysis of the characteristics of the amniotic fluid cultures in the high-virulence group revealed no features that distinguished the group of women who remained asymptomatic from those who developed symptoms (Table I). The most common high-virulence isolates were: Bacteroides bivius, 30; group B streptococci, 16; and anaerobic cocci, 14. Escherichia coli was isolated in three specimens, and S. aureus in six. Overall, U. urealyticum was isolated in 51% of the specimens tested and M. hominis in 26%. In the uterine infection group, the cultures of the patients who were delivered vaginally were similar to those of patients who were delivered abdominally.

Characteristics of patients in these three groups are shown in Table II. Although there were no significant differences in interval from rupture of membranes to collection or from onset of labor to collection, there were significant differences in the intervals from collection to delivery and in rate of cesarean section delivery. According to the data provided in Table II, 92% (11/12) of patients in the high-virulence group with cesarean section developed endometritis. In comparison, only 12% (6/48) of patients in the high-virulence group who had vaginal delivery developed endometritis, and 33% (16/48) developed fever only. Fully 54% (26/48) of patients in the high-virulence group with vaginal delivery remained asymptomatic. In the uterine infection group, the mean interval from collection to delivery was 4.8 hours for the six vaginal deliveries and 5.1 hours for the 11 abdominal deliveries (NS).

Features of the maternal and neonatal hospitalizations are shown in Table III. Among infants born to women who developed uterine infection, 50% (3/6) of

Table II. Characteristics of groups of patients

	Puerperal course							
Characteristic	Asymptomatic (n = 27)	P^t	Fever only (n = 16)	P^2	Uterine infection $(n = 17)$	P^{j}		
Age (yr)	24.2 ± 7.7	0.03	19.9 ± 4.8	0.04	24.7 ± 7.7	NS		
Parity	1.2 ± 2.0	0.05	0.3 ± 1.0	NS	1.2 ± 2.2	NS		
Gestational age (wk)	39.6 ± 2.5	NS	40.4 ± 2.0	NS	40.5 ± 2.1	NS		
Temperature in labor (°F)	98.5 ± 0.5	NS	98.7 ± 0.4	NS	98.8 ± 0.5	NS		
White blood cell count in labor (10 ³ /mm ³)	12.6 ± 7.8	NS	12.1 ± 5.5	NS	10.3 ± 0.7	NS		
Rupture of membranes to collection (hr)	15.8 ± 13.4	NS	13.2 ± 8.9	NS	16.9 ± 11.1	NS		
Labor to collection (hr)	8.1 ± 5.2	NS	7.6 ± 5.2	NS	8.8 ± 6.1	- NS		
Collection to delivery (hr)	2.2 ± 1.3	0.06	3.8 ± 3.2	0.10	5.2 ± 3.2	0.001		
		(NS)	— O.E	(NS)	J	0.001		
Cesarean section delivery (%)	3.7	NS	0	< 0.001	64.7	< 0.001		

Data are provided as mean \pm SD for all characteristics except cesarean section delivery. P¹, P², P³—See Table I.

Table III. Characteristics of maternal and neonatal hospitalizations

	Puerperal course							
Characteristic	Asymptomatic (n = 27)	P^{I}	Fever only $(n = 16)$	P ²	Uterine infection $(n = 17)$	P^{i}		
Maternal			<u>*************************************</u>	-1	·			
Temperature maxi- mum (°F)	98.8 ± 0.4	p < 0.001	100.0 ± 0.6	< 0.001	101.7 ± 1.2	< 0.001		
Hospital stay (days) Bacteremia	2.6 ± 0.8 0 of 0	NS	2.5 ± 0.5 0 of 0	< 0.001	7.9 ± 4.9 4 of 14	< 0.001		
Neonatal weight at birth (gm)	3267 ± 418	NS	3127 ± 331	< 0.02	3519 ± 589	NS		
Apgar score <7 at 1 minute, number (%)	1 (4)	NS	3 (19)	NS	7 (41)	0.003		
Apgar score <7 at 5 minutes, number (%)	0		0		1 (6)			
Suspected neonatal sepsis, number (%)	1 (4)	NS	1 (6)	< 0.001	6 (35)	< 0.001		
Neonatal hospital stay	2.6 ± 0.8	NS	2.6 ± 0.6	< 0.001	7.6 ± 4.8	< 0.001		

Data are provided as mean \pm SD for all characteristics except as noted. P^1 , P^2 , P^3 —See Table I.

those born vaginally and 36% (4/11) of those born abdominally had low 1-minute Apgar scores.

Comparative data. We were able to match successfully 40 pairs of patients. There were no significant differences in the matching characteristics: rupture of membranes to delivery interval $(16.4 \pm 4.0 \text{ versus})$ $16.9 \pm 7.7 \text{ hours}$, for the high- and low-virulence groups, respectively) and mode of delivery (both 15%). Furthermore, there were no significant differences in age, parity, gestational age, length of labor, white blood cell count in labor, or birth weight. Table IV shows the outcomes in the two groups. Interestingly, more neonates in the high-virulence group had Apgar scores <7 at 1 minutes than did the neonates in the low-virulence group (17.5% versus 2.5%, p = 0.025), but by 5 minutes of life, all neonates had Apgar scores ≥ 7 .

Comment

Previous investigations have provided a partial understanding of the relationship between bacteria in the amniotic fluid of parturient women and clinically evident infection. In a controlled study, Gibbs et al. reported that women with clinical intra-amniotic infection had $\geq 10^2$ cfu/ml in 81% of cases (42/52) compared with only 31% of control subjects (16/52) (p < 0.001). More than 10^2 high-virulence isolates per milliliter were found in 70% of samples of amniotic fluid from patients with intra-amniotic infection and in only 8% of samples from control subjects (p < 0.001). In a smaller, nonmatched series, Gravett et al. found bacteria in the amniotic fluid of 88% (14/16) of patients with clinical amniotic fluid infection and in that of 50% (11/22) of comparison patients (p < 0.01). Furthermore, of those

Table IV. Clinical outcomes of 40 matched pairs of patients

	High virulence			Low virulence only	
Outcome	No.	%	P	No.	%
Asymptomatic Fever only Endometritis Other infections	16 15 9	40 37.5 22.5	<0.01	27 10 1* 2†	67.5 25 2.5 5

^{*}This case was diagnosed 10 days post partum.

with positive cultures, bacterial growth on primary plates was $\ge 2 + \text{in } 13$ of 14 (93%) women with amniotic fluid infection, but in only two of 11 (18%) women in the comparison group (p < 0.0005). Only facultative organisms were isolated in the comparison group, whereas both facultative and anaerobic organisms were isolated from specimens of patients with amniotic fluid infection.

Four reports have noted that bacteria in the amniotic fluid of asymptomatic patients who underwent cesarean delivery commonly resulted in postoperative uterine infection. Gilstrap and Cunningham⁵ performed qualitative cultures on amniotic fluid collected transabdominally from 56 patients at cesarean section (all of whom had had ruptured membranes for 6 or more hours) and isolated bacteria in all, and endometritis developed in 95%. Cooperman et al.6 also performed qualitative cultures of amniotic fluid, amniotic membrane, and endometrium and found a similar correlation between positive cultures and endometritis. Using quantitative amniotic fluid cultures, Blanco et al.2 found ≥102 cfu of high-virulence bacteria per milliliter of amniotic fluid in 13 of 36 (36%) women at nonelective cesarean section. Twelve of these 13 (92%) developed endometritis, compared to nine of 23 (39%) women with negative cultures (p < 0.002). In specimens collected transcervically, D'Angelo and Sokol7 showed that postpartum endometritis developed in 92% (11/12) of women with ≥10° cfu/ml of any isolate, as compared to 47% (9/19) of women with $<10^4$ cfu/ml (p < 0.025). Also endometritis developed in 86% (12/14) of women with "streptococci," Bacteroides, enteric bacilli, or S. aureus, and in 47% (8/47) of women with none of these organisms (p = 0.025).

Few data are available on the relationship of amniotic fluid bacteria and complications after vaginal delivery. D'Angelo and Sokol⁷ found that endometritis developed in only two of 70 patients with vaginal delivery. However, these authors noted that an "elevated" fever index (defined as $>0.4^{\circ}$ C-hours) developed in 59% (29/49) of patients with $>10^{4}$ cfu/ml, but in only 33% (7/21) of patients with $<10^{4}$ ml (p = 0.05). An "elevated" fever index was observed in 72% (27/37) of women with

streptococci, *Bacteroides sp.*, or enteric bacilli, but in only 30% (10/33) with none of these organisms (p < 0.001).

Transient, self-limited, low-grade fever in the puer-perium occurs commonly. Calman and Gibson⁸ noted that 35% (954/2701) of women had a temperature >99.4° F on any occasion. Of these 954, 52% (499) were labeled as having pyrexia of no significance. More recently, Filker and Monif³ reported a temperature ≥38° C (100.4° F) in 65 of 1000 patients. Of 858 women with vaginal delivery, 33 (3.8%) had fever, which resolved spontaneously in 26 (78%). Of 142 patients who underwent cesarean section, 32 (22%) had exhibited one or more instances of a temperature ≥38° C, but in only nine of these 32 (28%) did it resolve spontaneously. These instances of fever have been attributed to breast engorgement, dehydration, atelectasis, etc., but the etiology of the fever has not been clearly demonstrated.

Descriptive data. In the present study, we extended the above-mentioned observations by analyzing the clinical course of 60 asymptomatic patients who had high-virulence bacteria in the amniotic fluid. These patients were considered to be at high risk for infection on the basis of the interval (usually >10 to 12 hours) from rupture of membranes to delivery. Three courses were evident: asymptomatic, fever only, and uterine infection. We observed no statistically significant differences among the amniotic fluid cultures of the three subgroups. It might be suggested that, because the groups were not large, real differences in one or more features existed, but were not detected. Overall, though, we believe that there is marked similarity, and in none of the features was there a "trend" in numbers or kinds of bacteria cultured.

Although many clinical features of the three subgroups were similar, there were significant differences in collection-to-delivery interval and in mode of delivery. We observed a progressive increase in collectionto-delivery interval from the asymptomatic group, to the fever-only group, to the uterine infection group. We did not determine, however, when the colony count of high-virulence bacteria first exceeded 10² per milliliter in a given patient and, subsequently, how long the total interval was with high-virulence bacteria in the

[†]One urinary tract infection and one wound infection.

amniotic fluid. Although the latter information might be exceedingly helpful, it would be difficult to obtain, by requiring an impractically large number of samples and a good deal of luck. Even though time from collection to delivery is not ideal, it is the best approximation we have, since there was no systematic bias in time of collection.

Distinguishing "fever only" from uterine infection may be largely subjective and especially difficult after cesarean section. Yet, even if we combined all patients with fever (those with and without signs of endometritis), the quantitative relationships remain the same.

Neonatal outcome was good in all groups, although the likelihood of a 1-minute Apgar score <7 and of clinically suspected sepsis was significantly greater in infants of mothers in the uterine infection group. Although suspected sepsis may have been influenced by the pediatricians' knowledge of maternal course, Apgar scores were assigned before any mother developed symptoms. A potential confounding variable in interpreting Apgar scores in the uterine infection subgroup was the large percentage (65%) of deliveries by cesarean section, but there was no difference in the rates of low Apgar scores between the group delivered abdominally and that delivered vaginally. By 5 minutes of life, nearly all infants were vigorous. Nevertheless, the high percentage of transiently depressed newborn infants of mothers who developed infection is striking

Comparative data. When 40 patients with high-virulence bacteria in the amniotic fluid were matched with patients with low-virulence bacteria in the amniotic fluid for interval from rupture of membranes to delivery and for mode of delivery, we found sign ficant differences in outcome. As we expected from previous work, especially with cesarean sections, patients in the high-virulence group had endometritis significantly more often than did the patients in the low-virulence group (p < 0.01). It was less expected that transient, self-limited, low-grade fever occurred commonly in both groups (37.5% versus 25%, NS). This observation may be interpreted in a number of ways. First, these instances of transient fever may have been unrelated to bacterial infection of the uterus. Traditionally, they have been attributed to breast engorgement, dehydration, atelectasis, etc. Second, it is possible that some of these instances of fever, perhaps those in the highvirulence group, were indeed due to a transient bacterial infection, whereas others, perhaps those in the low-virulence group, were due to other causes. Third, because not all of the high-virulence bacteria and the low-virulence bacteria have the same pathogenic potential, possibly errors were made in classifying them. Fourth, it is possible that these instances of fever were

caused by microbes, such as Chlamydia trachomatis, or viruses for which we did not perform cultures.

Conclusion. An intricate relationship exists between (1) type and numbers of bacteria in utero, the duration of bacteria in utero, and route of delivery and (2) the development of clinically evident infection. We offer the following conclusions. This study reconfirms the very high rate of endometritis (>90%) in patients who undergo cesarean section when high-virulence bacteria are already present in the amniotic fluid. Endometritis or fever is less common (46%) in patients who undergo vaginal delivery when high-virulence bacteria are present in the amniotic fluid, even though there is no discernible qualitative or quantitative difference in cultures. Although this study did not permit us to determine how long the high-virulence bacteria were present in the amniotic fluid before delivery, we did note a progressive and significant increase in the interval from collection (of fluid with high-virulence bacteria) to delivery, from the asymptomatic group, to the fever-only group, to the endometritis group. Since we are not aware of any systematic bias in collecting the amniotic fluid, the implication is that high-virulence bacteria were present for longer intervals in the patients with high-virulence bacteria who developed endometritis than in the asymptomatic patients. We observed "fever only" often in both groups and are unable to discern ts etiology.

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Congenital complete heart block and SSA antibodies: Obstetric implications

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Mothers with known or occult rheumatic disorders may be delivered of infants with congenital complete heart block. The more frequent use of ultrasonography during pregnancy now allows early detection of heart block in utero. The transplacental passage of SSA or SSB antibodies, of the IgG class, may mediate or be associated with immune damage to the fetal cardiac conduction system, as reported in our two patients. Maternal and/or newborn screening for SSA and SSB antibodies in selected patients permits an early presumptive diagnosis and will assist perinatal planning, particularly for immediate newborn cardiac pacemaker implantation. Early serologic detection of such antibodies may also assist family counseling of mothers at risk and should promote investigation of techniques to modify the immune status of these mothers. SSA- or SSB-positive maternal/fetal pairs should be prospectively managed by the obstetrician, neonatologist, and rheumatologist. (Am J Obstet Gynecol. 1985;152:655-8.)

Key words: Congenital complete heart block, SSA antibodies, obstetric implications

Congenital complete heart block is an occasional neonatal complication of occult or recognized maternal connective tissue diseases. By 1979, one investigation was able to review 67 cases of congenital complete heart block with associated maternal connective tissue disease. When the maternal connective tissue disease is recognized prior to delivery, systemic lupus erythematosus is the most frequent illness (38%) associated with congenital complete heart block; 25% of mothers have less specific rheumatic complaints, and 16% have rheumatoid arthritis. Overall, however, 70% of the mothers of infants with congenital complete heart block are asymptomatic and have only serologic abnormalities; some describe this as occult connective tissue disease, and recognition may be increased by a more detailed history.

Morquio first reported congenital complete heart block in 1901, and the first kindred of multiple affected children was described in 1928. By 1977, at least 14 kindreds of familial congenital complete heart block had been reported, and its poor prognosis in comparison with sporadic and adult-onset forms had been noted. Congenital complete heart block had previously been thought to occur only once in 20,000 pregnancies, but more common use of obstetric ultrasonography has led to anecdotal observations that congenital complete heart block may be more frequent. One fourth of cases are associated with structural cardiac malformations, but cause of the other 75% has remained obscure.³

In 1960, Wallgren and Agoria4 first postulated a maternal immunologic abnormality to explain the congenital complete heart block which was detected in three successive live births from one mother. By 1977, McCue et al.5 and Chameides et al.6 confirmed the association between maternal systemic lupus erythematosus and neonatal heart block. In recent years a somewhat artificial distinction between the neonatal lupus syndrome and congenital complete heart block has developed along subspecialty lines. The neonatal lupus syndrome almost always includes cutaneous lesions, while anemia, thrombocytopenia, abnormal serologic tests, and complete heart block are less common features. These infants are usually female, and with the exception of congenital complete heart block, the other findings generally clear within 2 to 6 months. The transplacental passage of an IgG antinuclear antibody has been presumed to cause neonatal lupus syndrome and congenital complete heart block, although a genetic or infectious etiology could not be entirely excluded.

In 1981, Franco et al.⁷ first reported a more specific association between neonatal lupus syndrome and maternal antinuclear antibodies directed against the SSA and SSB antigens in three maternal/infant pairs. The SSA and SSB antibodies (also known as Ro and La, respectively) are directed against two of a number of recently described saline-soluble ribonucleoprotein an-

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Reprint requests: B. H. Singsen, M.D., Associate Professor, Department of Child Health, University of Missouri Health Sciences Center, One Hospital Dr., Columbia, MO 65212. tigens from cell nuclei. Several of these antigen-antibody systems have important rheumatic disease associations, such as Sm antibodies with systemic lupus erythematosus, RNP antibodies with mixed connective tissue disease, Scl-70 with scleroderma, and SSA and SSB with Sjögren's syndrome; new information also indicates that antigens such as Sm and RNP may be components of the intracellular messenger RNA system.8

In the report from Franco et al.,7 the asymptomatic mothers remained well 3 years following delivery, except for continued serologic evidence of antinuclear antibody, rheumatoid factor, and SSA and SSB antibodies. At 2 to 3 years of follow-up, the neonatal lupus syndrome infants were serologically and clinically normal, but two of three were incidentally noted to have continuing atrioventricular heart block. Kephart et al.9 have described a fourth SSA-positive infant with neonatal lupus syndrome and congenital complete heart block. They also mentioned that the serum of another mother with systemic lupus erythematosus and the amniotic fluid from the 20-week abortus with congenital complete heart block both contained SSA and SSB antibodies. However, the fetal serum did not demonstrate SSA or SSB antibodies, perhaps indicating that the largest amount of maternal IgG antibody crosses the placenta during the third trimester. Weston et al.10 recently showed that seven of eight infants with neonatal lupus had speckled antinuclear antibody and SSA antibodies; four of eight had congenital complete heart block. The mothers were all SSA positive, but only two had overt rheumatic disorders.

The present report will re-focus the relationship between neonatal lupus syndrome, congenital complete heart block, and SSA antibodies and emphasize the implications for obstetric diagnosis and management. Only two early, brief notations of maternal rheumatic disorders and congenital complete heart block can be found in the obstetric literature.11, 12 Our patients confirm that clinically healthy pregnant women may have serologic evidence only of SSA antibodies and presumably pass these to the fetus in the second or third trimester and that the infant develops congenital complete heart block that can be detected in utero many weeks prior to the expected delivery date or during the perinatal period. Coordinated management and counseling for the SSA-positive woman who is pregnant or of childbearing age may begin with obstetrics and subsequently involve both pediatrics and rheumatology.

Case reports

Case 1. A 19-year-old woman with a 28-week pregnancy was referred in 1981 because of fetal bradycardia and irregular heart rhythm detected at her second obstetric evaluation. A fetal heart rate of 144 bpm had been noted 6 weeks earlier. There was no personal

or family history or physical evidence of any rheumatic disorder. A prior pregnancy had spontaneously aborted, without known cause, at 3 months' gestation. The remaining past medical and gynecologic history was unremarkable, and there were no other intrapartum complications. Evaluation by ultrasonography suggested a gestational age of 24 to 26 weeks, a fetal weight of 1000 gm, and a fetal heart rate of 48 bpm. The administration of intravenous isoproterenol to the mother did not increase the fetal heart rate; congenital complete heart block was suspected.

Maternal evaluation for laboratory evidence of rheumatic disorders included a normal complete blood count and erythrocyte sedimentation rate; the serum calcium level was 8.6 mg/dl and the magnesium level was 1.5 mEq/L. Immunologic screening revealed anti-SSA antibodies in a titer of 1:256; there were no antibodies directed against the SSB, DNA, RNP, Sm, Scl-70, or PM-1 antigens; rheumatoid factor and antinuclear antibody were not sought.

When the estimated gestational age reached 34 weeks, amniocentesis revealed a lecithin/sphingomyelin ratio of >4:1, suggesting fetal pulmonary maturity. Maternal serologic testing then included an antinuclear antibody titer of 1:80, normal serum IgG, IgM, and IgA levels, a positive test for Scl-70, SSA antibodies at 1:256, and a rheumatoid factor titer of 1:640. The serum C3 level was 76 mg/dl (normal 90 to 175), and the C4 level was 5 mg/dl (normal >13); there were no anti-DNA or anti-SSB antibodies present.

Elective cesarean section was performed after extensive consultation, in the hope of preventing further possible immunologic damage to the fetal cardiac conduction system; the mother's postoperative course was uneventful. The infant's birth weight was 2400 gm, and Apgar scores were 4 and 8. The cord blood revealed SSA antibodies, IgG of 733 mg/dl, a rheumatoid factor titer of 1:40, and a speckled antinuclear antibody titer of 1:40. Immediate electrocardiogram confirmed congenital complete heart block (rate 48 bpm), and weak inspiratory efforts led to intubation and respirator assistance. Isoproterenol raised the heart rate only to 60 bpm; a nitroprusside drip, begun at 42 hours of age because of worsening peripheral perfusion, was not effective. Hydrocortisone, 10 mg/kg/day, was administered in an attempt to control possible cardiac conduction system edema or continuing immune system-mediated damage. Extensive search for evidence of infection was negative. The blood pressure was constant at 60/40 mm Hg; however, renal perfusion and urine output were poor, and there was little response to furosemide. Fluid retention and hyperkalemia developed, and the heart rate dropped to 20 to 40 bpm and was irregular. The infant died at 84 hours of age. Postmortem examination limited solely to the cardiac conduction system was permitted. It revealed moderate fibrosis of the sinoatrial and atrioventricular nodes and the His bundle. There were no inflammatory infiltrates, and immunofluorescent staining of numerous serial sections was negative for IgG, IgM, IgA, IgD, C3, C4, and fibrin.

Case 2. A 25-year-old woman (gravida 3, para 2, abortus 1) was delivered of a normal female infant at term with Apgar scores of 8 and 9. There were no complications during pregnancy; monthly obstetric evaluations, beginning at 28 weeks, had revealed a normal fetal heart rate. Labor was prolonged at 42 hours, but the infant was in no distress when brought to the nursery. The mother had no personal or family history or physical evidence of any rheumatic disorder.

The infant became lethargic and displayed poor feeding by 20 hours of age. The heart rate, which had previously averaged 160 bpm, was noted to be 50 bpm; an electrocardiogram revealed complete heart block. Adequate peripheral pulses were present and neurological evaluation was normal. No other manifestations of the neonatal lupus syndrome were observed. A chest x-ray film revealed clear lung fields and moderate cardiomegaly. By 48 hours, the child had a heart rate of 36 bpm and moderate tachypnea. Despite fluid restriction, she developed congestive heart failure and by 57 hours of age was dusky, had progressive acidosis, and required isoproterenol to maintain peripheral perfusion. A permanent cardiac pacemaker was implanted; 12 hours later there was a significant decrease in body weight and liver size and increased urinary output.

Serologic evaluation of the mother and infant showed each to have SSA antibodies, in titers of 1:256 and 1:64, respectively. Rheumatoid factor, antinuclear antibody, and antibodies directed against the RNP, Sm, SSB, PM-1, Scl-70, and DNA antigens were all absent in both infant and mother. The infant was discharged, without further complication, on the twelfth hospital day. At 16 months of age she is developing normally, and pacemaker function is consistent at 120 bpm.

Comment

Our maternal-fetal pairs demonstrate that isolated SSA antibodies, probably of the IgG class,¹³ appear to cross the placenta and are associated with damage to the fetal cardiac conduction system. It is possible that the maternal and/or fetal SSA antibodies are only an immunologic epiphenomenon, or they may reflect an altered immune state induced by pregnancy in selected patients during the second or third trimester. We are currently assessing the frequency, by trimester, of SSA antibodies in asymptomatic pregnant women and the frequency of conversion from SSA negative to SSA positive in pregnant women with systemic lupus erythematosus, scleroderma, rheumatoid arthritis, and Sjögren's syndrome. This will allow a better understanding of the size of the populations at risk.

Practically speaking, however, it is the unexpected obstetric or perinatal diagnosis of congenital complete heart block in an otherwise normal pregnancy that requires our immediate attention. These mothers and infants may have only SSA antibodies, without evidence of other antinuclear antibodies, and isolated heart block

occurs in this situation. That placental transfer of SSA may be significantly associated with isolated congenital complete heart block has been confirmed by Scott et al.¹⁴

The immunogenetics relating maternal connective tissue diseases, SSA antibodies, and congenital complete heart block are not fully understood. Several brief reports suggest an association of DR3 and other linked HLA antigens in the mothers but not the infants. This may imply that maternal autoantibody production is related to genetic factors but that the neonatal cardiac conduction tissue injury is not.^{15, 16}

The cardiac histopathologic features in infants with congenital complete heart block have been described as interruption of the conduction system by fibrosis, fibrotic replacement of the sinoatrial and atrioventricular nodes, and calcification suggestive of earlier inflammation.9 These changes are similar to those found in our infant who died. Subendocardial fibroelastosis has also been detailed, including one report where seven of nine infants had "endomyocardial fibrosis" and three of three attempts to identify the sinoatrial node failed.1 Subendocardial calcium deposits have been noted in one infant.2 These recent histopathologic descriptions should be clearly distinguished from earlier studies of congenital complete heart block as a nosologic entity, which were without regard for the disease state of the mother.

We are aware of one fatal case of congenital complete heart block, with immunofluorescent evidence of IgG and C3 deposits within the vascular portion of the conduction system, as well as generalized chronic inflammatory cell infiltrates. However, both inflammatory infiltrates and immune deposits in the conduction system of the newborn infant with heart block may rapidly disappear and be replaced by fibrosis if the inciting antibody (or epiphenomenon) either disappears or is modified by treatment. Although the sinus node is found by 6 to 8 weeks of fetal age,5 fetuses who develop congenital complete heart block may have normal cardiac rate and rhythm as late as several hours after birth, or the congenital complete heart block may develop as early as 22 to 26 weeks. This suggests that the immunologic damage may occur early, develop slowly, or appear late.

In adults with systemic lupus, numerous forms of immunologically mediated cardiac damage may occur, but many have in common the fibrosis of conduction elements, much as may occur in the infant with congenital complete heart block.⁵ Complete atrioventricular block is a rare complication of adult systemic lupus erythematosus,¹⁷ but fibrosis of the conduction system is observed and is presumed to be due to its rich collagen content and its superficial location.

Unfortunately, overt maternal connective tissue dis-

ease may develop only months to years after the birth of an infant with congenital heart block. This emphasizes the need for a careful obstetric history of rheumatic complaints in any prospective or recent mother whose infant has developed congenital complete heart block.

Our experience and a review of the literature suggest several conclusions and recommendations: (1) The isolated presence of SSA antibodies at the time our patients developed congenital complete heart block and similar findings from other reports may suggest an etiologic role for SSA antibodies. (2) Pregnant mothers with clinical rheumatic disorders who are SSA positive may be at risk for having an infant with congenital complete heart block. (3) Women with systemic lupus erythematosus, scleroderma, rheumatoid arthritis, and Sjögren's syndrome who are considering becoming pregnant should be screened for SSA antibodies. (4) Similarly, significant numbers of women with vague musculoskeletal complaints are found to be SSA positive by antinuclear antibody profile. It may be important to educate these latter two groups about the possibility of congenital complete heart block as a pregnancy outcome and to recommend close obstetric observation.

The routine use of abdominal ultrasound now frequently permits early detection of congenital complete heart block, which should elicit rheumatologic consultation and immunologic screening. Management of the SSA-positive maternal-fetal pair is the complex question. The current postdelivery mortality rate for these infants approximates 25%. Early cesarean section to prevent further immune injury to the conduction system is one possibility, although our first experience suggests that the cardiac damage has long since occurred and that immediate cardiac pacing is needed. However. cesarean section to minimize fetal distress in the birth canal and to present the infant directly to a pediatric cardiologist who is prepared to initiate cardiac pacing might also be very helpful in selected cases. In this setting, a brief trial of labor to monitor the response of the fetal heart to stress may be appropriate.

Medical management of the SSA-positive pregnant mother by a rheumatologist also may be indicated. It is known that titers of SSA antibodies decrease during corticosteroid therapy.¹³ The utility of other methods to reduce or remove maternal SSA or SSB antibodies or their epiphenomena remains speculative. In summary, our experience suggests a critical need for early intra-

uterine detection, appropriate immunologic screening, and early cardiologic intervention for maternal/fetal pairs with or at increased risk for developing congenital complete heart block.

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Because her estrogen therapy shouldn't affect her blood pressure



In a three-month study,¹ none of the 184 patients receiving OGEN 1.25 experienced a significant rise in BP. About 15% of the 144 patients receiving conjugated estrogens (0.625 mg) experienced a significant rise of at least 15 mm Hg. That's a difference that could make a real difference for your patients who may be hypertensive or at risk of developing hypertension.

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Unlike conjugated estrogen tablets, scored OGEN tablets permit the use of half tablets. So you can adjust precisely to the minimum needed for symptom control. One more reason more doctors are considering OGEN. And more women are glad they are.

See adjacent page for brief summary.

Reference:
1. Wren BG, Routledge AD: The effect of type and dose of oestrogen on the blood pressure of post-menopausal women. Maturitas 5:135-142, 1983.

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OGEN®

ESTROPIPATE TABLETS, USP

WARNING:

1. ESTROGENS HAVE BEEN REPORTED TO INCREASE THE RISK OF ENDOMETRIAL CARCINOMA.

Three independent case control studies have shown an increased risk of endometrial cancer in postmenopaasel women exposed to exogenous estrogens for prolonged periods. 1-3 This risk was independent of the other known risk factors for andometrial cancer. These studies are further supported by the finding that incidence rates of endometrial cancer have increased sharply since 1989 in eight different areas of the United States with population-based cancer reporting systems, an increase which may be related to the rapidly expanding use of estrogens during the last Jecade.

The three case control studies reported that the risk of endometrial cancer in estrogen users was about 4.5 to 13.9 times greater than in nonusers. The risk appears to depend on both duration of treatment and on estrogen dose. 2 In view of these findings, when estrogens are used for the treatment of menopaeaal symptoms, the lowest dose that will control symptoms should be discontinued as soon as possible. When prolonged treatment is medically indicated, the patient should he reassessed on at least a semianoual basis to determine the need for continued therapy. Although the evidence must be considered preliminary, one study suggests that cyclic administration of low doses of estrogen may carry less risk than continuous administration, 3 it therefore appears prudent to utilize such a regimen.

Close clinical surveillance of all women taking estrogens is important. In all cases of undisgnosed persistent or recurring abnormal vapinal bleeding, adequate diagnostic measures should be undertaken to rule out malignancy.

There is no evidence at present that "natural" estrogens are more or less hazardous than "synthetic" estrogens at equiestrogenic doses.

or less hazardous than "synthetic" estrogens at equiestrogenic doses.

or less hazardous than "synthetic" estrogens at equiestrogenic doses.

2. OGEN SHOULD NOT BE USED DURING PREGNANCY.

According to some investigators, the use of female sex hormones, both estrogens and progestogens, during early pregnancy may seriously damage the offspring. Studies have reported that females exposed in utero to diethylstifibestrol, a non-steroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. So in one of these studies, this risk was estimated as not greater than 4 per 1000 exposures. Truthermore, there are reports that a high percentage of such exposed women (from 30 to 90 percent) have been found to have veginal adenosis, 9-12 epithelial changes of the vagina and cervix. Although these reported changes are histodigically benign, the investigators have not determined whether they are precursors of adenocercinoma.

Several reports suggest an association between intrauterine exposure to female sex hormones and congenital anomalies in the offspring, including heart defacts and limb reduction defacts. 3-19 for ease control study!⁴⁶ estimated a 4,7 fold increased risk of limb reduction defacts in infants exposed in utero to sex hormones (oral contraceptives, hormone withdrawal tests for pregnancy, or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb reduction defects in exposed fetuses is somewhat less than 1 per 1000.

In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habit al abortion. DEEN has not been studied for these uses, and therefore should not be used during pregnancy. There is no evidence from wall controlled studies that progestogens are effective for there uses.

If OEEN lestropipate tatlats) is used during pregnancy, or if the patient becomes pregnant while taking this drug, she should be epprised of the potential risks to the fetu 2. OGEN SHOULD NOT BE USED DURING PREGNANCY.

- INDICATIONS AND USAGE
 The cyclic administration of OGEN (estropipace tablets) is indicated for the treatment of estrogen deficiency associated with (See "DUSAGE AND ADMINISTRATION" section):

 1. Moderate to severe vasamotor symptoms of menopause, (There is no evidence that estrogens are effective for nervous symptoms or depression which might occur during menopause, and they should not be used to treat these conditions.)
- Atrophic vaginitis.
- Kraurosis vulvae.

3. Knurosis vulvae.
4. Female hypogonadism.
5. Female castration.
6. Primary ovarian failure.
0GEN (ESTROPIPATE TABLETS) HAS NOT BEEN TESTED FOR EFFICACY FOR ANY PURPOSE DURING PREGNANCY. SINCE ITS EFFECT
UPON THE FETUS IS UNKNOWN, IT CANNOT BE RECOMMENDED FOR
ANY CONDITION DURING PREGNANCY (SEE BOXED WARNING).

CONTRAINDICATIONS

DEEN should not be used in women with any of the following conditions:

1. Known or suspected cancer of the breast.

2. Known or suspected suppen dependent neoplasia.

3. Known or suspected pregnancy (See Boxed Warning).

4. Undiagnosed ehoromal genital bleading.

5. Active thromobophlebits or thromboembolic disorders.

6. A past history of thrombophlebits, thrombosis, or thromboembolic disorders associated with previous sectione use

- ders associated with previous estrocen use

WARNINGS

WARNINGS

1. Induction of malignant neoplasms. Long-term continuous administration of natural and synthetic estrogens in certain animal species has been reported by some investigators to increase the frequency of carcinomas of the breast, cervix, vagina, and liver. There is now evidence that extraogens increase the risk of carcinoma of the endometrium in humans. (See Boxed Warning).

At the present time there is no satisfactory evidence that estrogens given to postmenopausal women increase the risk of cancer of the breast, "although a recent long-term followup of a single physician's practice has raised this possibility!" Therefore, caution should be exercised when administering estrogens to women with a strong family history of breast cancer or who have breast nodules, fibrocystic disease, or abnormal marmograms. Careful breast examinations should be performed periodically.

2. Gall bladder disease. A recent study has reported a 2 to 3-fold increase in the risk of surgically confirmed gall bladder disease in women receiving post-

menopausal estrogens, 17 similar to the 2-fold increase previously noted in users of oral contraceptives: 10 22 in the case of oral contraceptives the increased risk appeared after two years of use, 22 3. Effects similar to those caused by estrogen-progesto; an oral contraceptives. There are several serious adverse effects of oral contraceptives, most of which have not, up to now, been documented as consequences of postmenopausal estrogen therapy. This may reflect the comparatively lew doses of estrogens used in postmenopausal women. It would be expected that the larger doses of estrogen used to treat postpartum breast engorgement would be more likely to result in these adverse effects, and, in fact, it has been shown hat there is an increased risk of thrombosis in women receiving estrogens for assipartum breast engorgement. 2021

2. Thramboembolic disease. It is now well established theausers of oral contraceptives have an increased risk of various thromboembolic and thrombosit vascular diseases, such as thrombophlebitis, pulmonary embalism, stroke, and myocardial infarction. 22 22 Cases of retinal thrombosis, mose text intrumbosis, and optic neurits have been reported in oral contraceptive users. There is evi-

myocardial infarction.³²⁻²⁹ Cases of relinal thrombosis, mase teric thrombosis, and optic neurits have been reported in oral contraceptive users. There is evidence that the risk of several of these adverse reactions is relied to the disc of the dng. ³⁰⁻³¹ An increased risk of post-surgery thromboent-dir complications has also been reported in users of oral contraceptives. ³⁰⁻³¹ if -assible, estrogen should be discontinued at least 4 weeks before surgery of the type associated with an increased risk of thromboenbolism; it should also be discontinued during periods of prolonged immobilization.

While an increased rate of thromboenbolic and thrombotic-disease in post-menopuscul users of estrogens has not been found ¹⁷⁻³⁴ this does not rule out the possibility that such an increase may be present or that subgroups of women who have underlying risk factors or who are receiving relatively large doces of estrogens may have increased risk. Therefore estrogens shoul not be used in persons with active thrombophlebits or thromboembolic discraters, and they should not be used in persons with a history of such disorders in-association with estrogen use. They should be used with caution in vatients with errebral vascular or coronary artery disease and only for those ir whom estre ens are clearly needed.

Large doses of estrogen (5 mg conjugated estrogens per day, comparable to

needed.

Large doses of estrogen (5 mg conjugated estrogens per day , comparable to those used to treat cancer of the prostate and breast, have been shown in a large prospective clinical trial in men²⁵ to increase the risk of nonfate' myocardial infertion, pulmonary embolism and thrombophiebiris. When estrogen doses of this size are used, any of the thromboembolic and thrombotic adver e effects associated with oral contraceptive use should be considered a clear risk.

b. **Hapatic adenoma.** Benigh hepatic adenoma: appear to be associated with the use of oral contraceptives.**30-90 Although benigh, and rare, the semay nupture and cause death through intraabdominal hemorrhage. Such lesi as have not yet been reported in association with other estrogen or progestogen preparations but benut be considered in estrogen asers having abdomatial pain and tendemess, abdominal mass, or hypovolemic shock. Hepatocellular carcinom. has also been reported in women taking estrogen-containing oral contraceptives.** 77 The relationship of this malignancy to these drugs is not known at this time.

c. **Elevated blinda pressure.** Increased blood pressure is no uncommon in women using oral contraceptives.** There is now a report that this "Tay occur with use of estrogens in the mencpause** and blood pressure shoul. be monitored with estrogen use, especially bigh dosses are used.

d. **Glucose tolerance.** A worsening of glucose tolerance has seen observed in a significant percentage of patients on estrogen-containing oral ontraceptives.** for this reason, diabetic patients should be carefully observed while receiving estrogen.

4. **Moneraltemia.** Administration of estrogens raav lead to severe howercal-

estrogen.

4. Hypercalcemia. Administration of estrogens may lead to severe hypercalcemia in patients with breast cancer and bone metastases.

PRECAUTIONS

PRECAUTIONS

1. A Compeler medical and family history should be taken prict to the initiation of any estrogen therapy. The pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen, and polivic organs, and should include a Papanicolau smear. As a general rule, estrogen should not be prescribed for longer than one year without an ther physical examination belong referred. examination being performed.

examination being performed.

2. Fluid retention — Estrogens may cause some degree of f⊥id retention.

Therefore, patients with conditions such as epilepsy, migraine, ad cardiac or renal dysfunction, which might be influenced by this factor, require areful obser-

Certain patients may develop undesirable manifestations of excessive estrogenic stimulation, such as abnormal or excessive uterine bleeding mastodynia.

trogenic stimulation, such as abnormal or excessive uterne bleeding mastodynia, etc.

4. Oral contraceptives appear to be associated with an incressed incidence of mental depression. 22 Although it is not clear whether this is die to the estrogenic or progestogenic component of the contraceptive, patients with a history of depression should be carefully observed.

5. Preexisting uterine leionnymantal may increase in size during estrogen use.

6. The pathologist should be advised of the patient's use of estring the theory when relevant specimens are submitted.

7. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence of jaundice while receiving estrogen-chaining oral contraceptive therapy. If jeundice develops in any patient receiving strogen, the medication should be discontinued while the cause is investigated.

8. Estrogens may be poorly metabolized in patient with impaired liver function and they should be dusinistered with caution in such patients.

9. Because estrogens influence the metabolism of calcium and chappionus, they should be used with caution in patients with menabolic bone: sessess that are associated with hypercalcemia or in patients with menabolic bone: sessess shed are associated with hypercalcemia or in patients with renal insufficiency.

8. Information for the Patient. See text on Patient Package Insert x47ch appears after PHYSICIAN HEFERENCES.

C. Drug Interactions. The concomitant use of any drugs which can include hepatic.

after PHYSICIAN REFERENCES.
C. Drug Interactions. The concomitant use of any drugs which can inclice hepatic microsomal enzymes with estrogens may produce estrogen levels which are lower than would be expected from the dose of estrogen administered. The use of broad spectrum antibioties which profoundly affect intestinal flore may influence the absorption of steroidal compounds includin the estro-

gens.
Diabetics receiving insulin may have increased insulin requirement

Diabetics receiving insulin may have increased insulin requirements when receiving astrogens.
Laboratory Test Interference, Certain endocrine and liver function tests may be affected by estrogen-containing or all contraceptives. The following similar changs may be expected with larger doses of estrogen:

a. Increased sulfobromophihalein retention.
b. Increased sulfobromophihalein retention.
b. Increased onephipshine-induced platelet aggregability.
c. Increased they of the increased through bring increased distribution to the proof of the proof o

- Reduced serum folate concentration.

g. Reduced serum folate concertration.

h. Increased serum triglyceride and phospholipid concentration.

C. Carcinogenesis. Studies have shown an increased risk of endomercal cancer in postmenopausal women exposed to exogenous estrogens for prolong if periods (see Boxed Warning). At the present time there is no conclusive excence that estrogens given to postmenopausal women increase the risk of caneer of the breast17-46-17 There are, however, a few retrospective studies which suggest a small but statistically significant increase in the risk factor for breast cancer

among these women.18,42,44 (See "WARNINGS" section.)

Bregnancy, Pregnancy Category X. See "CONTRAINDICATIONS" section and Boxed Warning.

F. Nursing Mothers. Estrogens have been reported to be excreted in human breast milk. Caution should be exercised when OGEN is administered to a nursing

woman.

G. Pediatric Use. Because of the effects of estrogens on epiphyseal closure, they should be used judiciously in young patients in whom bone growth is not complete.

ADVERSE REACTIONS

ADVENSE FIGURE 10093 (See Warning regarding reports of possible induction of neoplasia, unknown effects upon the fetus, increased incidence of gall bladder cisease, and adverse effects upon the fetus, increased incidence of gall bladder cisease, and adverse effects upon the fetus, increased incidence of gall bladder cisease, and adverse effects upon the fetus, increased incidence of gall bladder cisease, and adverse effects upon the fetus increased incidence of gall bladder cisease, and adverse effects upon the fetus increased incidence of gall bladder cisease, and adverse effects upon the fetus increased incidence of gall bladder cisease, and adverse effects upon the fetus increased incidence of gall bladder cisease, and adverse effects upon the fetus increased incidence of gall bladder cisease, and adverse effects upon the fetus increased incidence of gall bladder cisease, and adverse effects upon the fetus incidence of gall bladder cisease, and adverse effects upon the fetus incidence of gall bladder cisease, and adverse effects upon the fetus incidence of gall bladder cisease, and adverse effects upon the fetus incidence of gall bladder cisease, and adverse effects upon the fetus incidence of gall bladder cisease, and adverse effects upon the fetus incidence of gall bladder cisease and gall bladd tects upon the fetus, increased dicidence of gall bradder cleases, and adverse effects similar to those of oral contraceptives, including thromboembolism). The following additional adverse reactions have been reported with estrogenic therapy, including oral contraceptives:

1. **Bonitourinary system** thromboembolisms** thromboembolis

Dysmenorrhea. Amenorrhea during and after treatment.

Panentines during and area reasoners.

Change in cervical eversion and in degree of cervical secretion.

Breakthrough bleeding, spotting, change in menstrual flow.

Premenstrual-like syndrome.

Premenstrual-like syndrome.

2. Breast.
Tenderness, enlargement, secretion.
3. Gastrointestinal.
Cholestatic jaundice.

Vomiting, nausea. Abdominal cramps, bloating. 4. Skin

Hemorrhagic eruption. Erythema nodosum. Erythema multiforme. Hirsutism.

Chloasma or melasma which may persist when drug is discontinued.

Loss of scalp hair. 5. Eves Steepening of comeal curvature. Intolerance to contact leases.

Intolerance to contact lenses.
6. CNS.
Chorea.
Mental depression.
Migraine, dizziness, headache.
7. Miscellaneous.
Aggravation of porphyria.

Edema. Reduced carbohydrate tolerance.

Increase or decrease in weight. Changes in libido.

Numerous reports of ingestion of large doses of estrogen-containing oral contra-ceptives by young children indicate that serious ill effects do not occur. Overdos-age of estrogen may cause nausea and withdrawal bleeding may occur in

DOSAGE AND ADMINISTRATION

Given cyclically for short-term use: For treatment of moderate to severe vasomotor symptoms, atrophic vaginitis,

For treatment of moderate to severe vazomotor symptoms, atrophic vagmits, or fraurosis vulvae associated with the menopause.

The lowest dose that will control symptoms should be chosen and medication should be discontinued as promptly as puss ble!

Administration should be vycic (e.g., 3 weeks on and 1 week off).

Attempts to discontinue or taper medication should be made at 3 to 6 month

Usual dosage ranges:

Vasomotor symptoms — One OGEN .625 (estropipate) Tablet to one OGEN 5 Tablet per day. The lowest dose that will control symptoms should be chosen. If the patient has not menstruated within the last two months or more, cyclic administration is started arbitrarily, if the patient is mentruating, cyclic administration is started on day 5 of bleeding.

Atrophic vaginitis and kraurosis vulvae — One OGEN 625 Tablet to one OGEN 5 Tablet daily, depending upon the tissue response of the individual patient. The lowest dose that will control symptoms should be chosen. Administer cyclically.

2. Given cyclically:
Female hypogonadism: female castration, primary ovarian failure. Usual dosage ranges:

Female hypogonadism; female castration, primary ovarian failure.

Isual dosage ranges:

Female hypogonadism — A deily dose of one OGEN 1.25 Tablet to three

OGEN 2.5 Tablets may be given for the first three weeks of a theoretical cycle,
followed by a rest period of eight to ten devs. The lowest dose that will control
symptoms should be chosen. In bleeding does not occur by the and of this period,
the same dosage schedule is repeated. The number of courses of estrogen therapy necessary to produce bleeding may vary depending on the 'esponsiveness of
the endometrium. If satisfactory withdrawal bleeding does not occur, an oral progestogen may be given in addition to estrogen during the third week of the cycle.

Female castration and primary ovarian failure — A deily dose of no

OGEN 1.25 Tablet to three OGEN 2.5 Tablets may be given for the first three
weeks of a theoretical cycle, followed by a rest period of eight to ten days,
dayst dosage upward or downward according to severity of symptoms and response of the patient. For maintenance, adjust dosage to lowest level that will
provide effective control.

Treated patients with an intact uterus should be monitored closely for signs of
endometrial cancer and appropriate diagnostic measures shoulc be taken to rule
out malignancy in the event of persistent or recurring abnormal vaginal bleeding.

HOW SUPPLIED

HOW SUPPLIED OGEN (estropiate tablets, USP) is supplied as DGEN. 825 (0.75 mg estropiate), yellow tablets, NDC 0074-3943-04, DGEN 1.25 (1.5 mg estropiate), peach-colored tablets, NDC 0074-3948-04; DGEN 2.5 (3 mg estropipate), blue tablets, NDC 0074-3951-04; and OGEN 5 (8 mg estropipate), light green tablets, NDC 0074-3951-104; and OGEN 5 (8 mg estropipate), light green tablets, NDC 0074-3951-13, Tablets of all four dosage levels are standardized to provide uniform estrone activity, and are grooved (Divide-Tab®) to provide dosage flexibility, All tablet sizes of DGEN are available in bottles of 100.

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Maternal heart rate in pregnancy

James F. Clapp III, M.D.

Burlington, Vermont

Serial morning heart rates were obtained from 10 women runners prior to and during pregnancy. By 8 weeks' menstrual age, maternal heart rate had risen 8 bpm with an overall pregnancy-associated increase of 16 bpm. Potential mechanisms are discussed. (Am J OBSTET GYNECOL 1985;152:659-60.)

Key words: Heart rate, pregnancy

It is well known that maternal heart rate rises 10 to 20 bpm during pregnancy. However, the serial data available have not been obtained under basal, stressfree conditions and it is unclear how early the observed increase actually begins.2 This report details the serial -changes in maternal heart rate in a group of 10 women runners prior to and during pregnancy under basal, stress-free conditions.

Methods

Each subject used a portable heart rate monitor (Insta-pulse Biosig, Inc., Montreal, Canada) to obtain her pulse in the lateral position on awakening each morning for at least I month prior to conception and throughout pregnancy. All data were normalized to the date of the last menstrual period, and individual weekly averages were calculated. Pregnancies were dated by means of menstrual records and confirmed by β-subunit assay for chorionic gonadotropin within 48 hours of the first missed menstrual period and early ultrasonic examination. Each monitor was precalibrated against an electrocardiographic trace and, at resting heart rate level, was within 2 bpm of the rate determined by electrocardiography.

Results

The data are detailed in Table I and Figs. 1 and 2. Note (Fig.1) that in each instance the maternal heart rate level increased by the time of the first missed menstrual period and showed no consistent change over the ensuing 4-week interval. The mean increase (Fig. 2, Table I) was 7 bpm at 4 weeks' menstrual age and 8 bpm at 8 weeks' menstrual age. A similar change was not observed in the preceding menstrual cycle. Thus, by 8 weeks (Table I), 50% of the maximum increase

PULSE RATE (bpm) 70 60 8 **PMP** LMP TIME

Fig. 1. Changes in resting maternal heart rate in the individual subjects at 4-week intervals normalized for the onset of the last menstrual period (LMP) and beginning during the previous menstrual period (PMP).

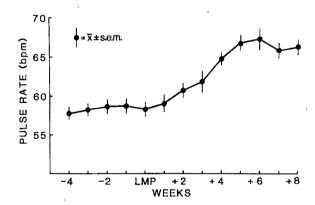


Fig. 2. Average weekly morning pulse rate in the 10 subjects prior to and during the first 8 weeks of pregnancy. All data normalized for the onset of the last menstrual period (LMP) and presented as the mean ± SEM.

had already occurred. This initial abrupt increase was followed by a more gradual progressive rise as pregnancy continued, which plateaued after the thirty-second week.

Comment

The overall increase of 16 bpm observed in this study is in agreement with most of the published literature.2 However, the fact that the increase begins abruptly very

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Table I. Maternal heart rate prior to and during pregnancy

,		Weeks' gestation (menstrual age)									
	-4	Last menstrual period	4	8	12	16	20	24	28	32	36
Maternal heart rate* (bpm)	57.8 ± 0.9	58.3 ± 0.9	64.8 ± 0.9	66.3 ±1.0	66.4 ±1.2	63.7 ±1.9	72.2 ±2.1	72.0 ± 1.7	72.4 ± 1.7	73.8 ± 1.8	74.0 ±2.2

^{*}Data presented as the mean \pm SEM.

early in pregnancy was unanticipated and suggests a hormonal mechanism not normally active during a menstrual cycle in which conception does not occur. From what is known in regard to the endocrinologic factors in early pregnancy, the initial change may be linked to the production of chorionic gonadottopin, with the later gradual increase being related to the vascular changes which accompany placental and fetal growth.

This work would not have been possible without the help and dedication of the volunteers from the University of Vermont College of Medicine.

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Second-look laparotomy after chemotherapy in the management of ovarian malignancy

Lynda R. Smirz, M.D., Frederick B. Stehman, M.D., Thomas M. Ulbright, M.D., Gregory P. Sutton, M.D., and Clarence E. Ehrlich, M.D.

Indianapolis, Indiana

Eighty-eight patients with ovarian malignancy underwent second-look laparotomy as part of their plan of management at Indiana University Hospital. Thirty-five patients (39.8%) were found to have no gross or microscopic evidence of persistent neoplasm, and an additional 16 (18.2%) had only microscopic tumor. Patients with initial Stage III or IV disease were less likely to have negative findings at laparotomy than were patients with Stage I or II disease. A complete initial cytoreductive operation (residual disease = 0 cm) correlated positively with negative findings at laparotomy. Endometrioid histologic findings were associated with a favorable condition at the second look, but tumor grade was associated with superior survival only for patients with grade 0 disease. Eight of 30 patients (26.7%) with invasive carcinoma and negative findings at second-look laparotomy have had tumor recurrence (2 to 63 months), and five of eight have died. Intraperitoneal chromic phosphorus salvage therapy for patients with microscopic disease was promising, with eight of 15 treated patients (53.3%) alive after therapy, with no evidence of disease from 16 to 56 months after the second look. Second-look laparotomy has been the major determinant of continued or alternative therapy for patients with ovarian malignancy. Second-look laparotomy has been incorporated into the standard management plan without a formal clinical trial. The need for a second look may be reduced by identifying patients who do not benefit from the procedure. Patients with persistent disease confirmed by noninvasive means can continue therapy without laparotomy. There is also a subgroup of patients with a favorable prognosis whose therapy could be safely discontinued without laparotomy. (AM J OBSTET GYNECOL 1985;152:661-8.)

Key words: Ovarian, carcinoma, laparotomy

"Second-look" laparotomy, i.e., planned exploratory laparotomy for patients who respond to an established course of chemotherapy, has gained acceptance as a standard procedure for patients with ovarian malignancy in order to assess the need for further therapy.1.5 Most patients with ovarian cancer will require some postoperative adjuvant therapy, which quite commonly is chemotherapy. As newer combination regimens are consistently able to produce a high percentage of long-term responses, there is an increasing number of patients whose disease status and need for continued therapy can only be evaluated by invasive means. 6.7 This problem is compounded by an aggressive cytoreductive operation which results in many patients starting chemotherapy without clinically measurable disease. Prolonged therapy with alkylating agents is recognized as

being associated with an increased risk for the development of nonlymphocytic leukemia.8 Hence, therapy should be terminated as soon as possible.

This report reviews the experience with second-look laparotomy in the management of patients with ovarian malignancy at Indiana University Hospital, in an effort to redefine the role of this operation.

Material and methods

From January 1, 1974, to December 31, 1982, 445 patients with ovarian malignancy were referred to the Section of Gynecologic Oncology of the Department of Obstetrics and Gynecology at Indiana University Hospital for treatment. Of these 445 patients, 88 underwent second-look laparotomy, defined as follows. (1) The diagnosis of ovarian cancer had been previously established at laparotomy. (2) A prescribed course of adjuvant therapy had been completed. (3) Patients had no clinical evidence of disease prior to second-look laparotomy. In no patient was exploration done solely to debulk known persistent disease. Patients were evaluated prior to operation, usually as inpatients, in an attempt to identify clinically covert disease. During the years of this study, there was considerable evolution in the preoperative radiographic evaluation.

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Table I. Characteristics of the patients

Age (yr)	
≤20	1
21-30	7
31-40	10
41-50	28
51-60	22
61-70	. 18
>71	2
FIGO stage	-
I	8
II	8
III	57
IV	11
Recurrent	4
	4
Histologic grade	c
0 1	6
	19
2	36
3	27
Cell type	
Serous	45
Adenocarcinoma (not otherwise	17
specified)	
Endometrioid	8
Mucinous	4 , 5 .
Clear cell	5 .
Low malignant potential	. 6
Other	3
Largest residual (cm)	
0	. 28
1	13
2	11
$\frac{-}{3}$	6
4-6	5 .
>6	15
Unknown	10

All operations were performed or directed by one of the senior surgeons (F. B. S. or C. E. E.). With the patient under general anesthesia, the operation was performed through a vertical midline incision. After the abdomen had been entered, washings from the pelvis, lateral gutters, and right subdiaphragmatic space were obtained for cytologic examination. Any visible disease or suspicious areas were biopsied for frozen section. If persistent disease was documented, the procedure was terminated. If no disease was visible, multiple biopsy specimens were obtained. Sites of biopsy included the right hemidiaphragm, pelvic peritoneum, lateral gutters, omentum, intestinal serosa, and any adhesions. Later in the series, pelvic and para-aortic nodal biopsy specimens were taken, even if the nodes were not enlarged. If not previously performed, oophorectomy, hysterectomy, and omentectomy were carried out. Prior to closure of the anterior abdominal wall, a Hemovac drain was placed along the right paracolic gutter with its end above the liver, and was brought out through a stab wound in the right flank. The drain provided an access route for intraperitoneal chromic phosphorus, if indicated.

All tissues were examined by a faculty member of

the Surgical Pathology Department. For this review, slides from the original operations were obtained in all cases and were reviewed by a single pathologist with a special interest in gynecologic pathology (T. M. U.). In each case, classification was made as to the histologic type of tumor, according to the criteria of the World Health Organization modified by Czernobilsky.9 Furthermore, each neoplasm was assigned a grade from 0 (equivalent to a tumor of low malignant potential) to 3 (poorly differentiated carcinoma), depending upon the extent of differentiated structures (architectural grade) as described by Czernobilsky for the grading of serous neoplasms. The nuclear grade was also assessed, by means of a method used for the evaluation of endometrial carcinoma10 and was found to correlate quite closely with the architectural grade. In the few cases that showed a difference of one grade between the architectural and nuclear grades, the higher of the two grades (almost always the nuclear grade) was assigned to the case. The grade was assigned irrespective of the presence or absence of metastatic disease.

The age range of the 88 patients was from 13 to 72 years (median, 50 years). Characteristics of the patients are listed in Table I. There were four patients who started chemotherapy at the time of tumor recurrence. The original stages had been I, I, II, and III. At review, six patients who had tumors originally classified as grade 1 had them reclassified as grade 0, tumors of low malignant potential. The age range of these six patients was from 28 to 50 years (median, 36.5 years). Their FIGO (International Federation of Gynecology and Obstetrics) stages were: I = two patients, II = one patient, and III = three patients. The epithelial adenocarcinomas predominated. There was one immature teratoma; one mixed germ cell tumor with elements of embryonal carcinoma, endodermal sinus tumor, and choriocarcinoma; and one mixed müllerian tumor.

The initial operation was performed by the referring physician prior to referral in most of the cases (69/88). There were 10 patients in whom the volume of residual disease was not described in the operative notes, nor coulc it be determined by a review of their charts. Seven of these patients had total hysterectomy and bilateral salpingo-oophorectomy; one had bilateral salpingo-oophorectomy; and two had unilateral salpingo-oophorectomy. None had clinically measurable disease at the beginning of therapy.

Postoperative chemotherapy was administered to 87 patients, and one patient received intraperitoneal chromic phosphorus. Cisplatin-based combinations were used in 53 patients (60.2%), and single alkylating agent therapy in 25 patients (28.4%). Nine patients received other chemotherapeutic regimens. Of the patients who received cisplatin therapy for invasive epithelial carcinoma, 96% (50/52) had Stage III or IV

Table II. FIGO stage versus therapy (epithelial carcinomas)

•	Stage I	Stage II	Stage III	Stage IV	Recurrent
Single alkylating agent	5	3	9	2	0
Cisplatin combination	0	2	40	7	3
Other chemotherapy	0	1	4	2	. 0

or recurrent disease, compared to only 58% of the patients who received an alkylating agent and 86% of the patients treated by other regimens, thus reflecting an institutional selection bias (Table II). Seventeen patients (21.8%) received eight or fewer cycles of therapy. Fortysix patients (59.0%) received between nine and 12 cycles of therapy. Fifteen patients (19.2%) received more than 12 cycles.

Results

Operative morbidity was minimal (Table III). Post-operative stay ranged from 3 to 22 days (median, 11 days). In most cases, further therapy, if indicated, was administered before discharge. The number of patients who received transfusion reflected, in part, postchemotherapy anemia that led to a liberal transfusion policy. There were no operative deaths.

In 35 patients (39.8%), no gross or microscopic disease was found at laparotomy. Microscopic disease or ly (biopsy or washing) was found in 16 patients (18.2%), and gross disease was apparent in 37 patients (42.0%).

The findings among the six patients with tumors of low malignant potential were: negative, three patients; microscopic positive, two patients; and grossly positive, one patient. Two of the three patients with nonepithelial tumors had negative findings at laparotomy, and one patient had gross disease. Analysis of risk factors is based on the patients who received therapy for invasive epithelial carcinoma.

Patients with initial Stage I or II disease were more likely to have negative findings at the second look than were patients with initial Stage III or IV or recurrent disease (9/12 = 75% versus 21/67 = 31.3%, p = 0.006). Table IV summarizes the correlation of findings at the second look with age, stage, grade, histologic type, residual tumor, and chemotherapy. Patients with no residual disease at the completion of the initial operation were more likely to have negative findings than were patients with residual disease (15/20 = 75.0% versus 15/59 = 25.4%, p = 0.0001).

Histologic grade did not correlate with findings at second-look laparotomy. However, there were seven instances of negative findings at laparotomy among the eight patients with endometrioid tumors. Neither the chemotherapy regimen administered nor the number of courses of chemotherapy correlated with the findings at second-look laparotomy. If single alkylating agent therapy is compared to cisplatin combination

Table III. Operative morbidity

Operative complications	No.
Blood transfusion	
≤2 (units)	11
>2 `	4
Wound infection	9
Urinary tract infection	8
Pneumothorax	1. 1
Bowel laceration	1
Gastrointestinal bleeding	l

therapy for only those patients with Stages III and IV and recurrent disease, the alkylating agent regimen produced negative findings at laparotomy in only two of 14 patients (14.3%), whereas the cisplatin combination produced negative findings at laparotomy in 17 of 50 patients (34%) (NS, p = 0.13).

In the 53 patients who had positive findings at the second look, persistent disease was documented most often in the pelvic peritoneum. Abdominal peritoneal surfaces (bowel serosa, lateral gutters, diaphragm, omentum), ovary, lymph nodes, and liver were other sites of documented persistence. Five of the 16 patients in the group with microscopic residual disease had positive cytologic washings as the only evidence of disease.

All patients were followed up either until the time of death or for a minimum of 12 months after secondlook laparotomy. Median follow-up was 24+ months after the second look (37+ months after diagnosis). Of the 30 patients with invasive epithelial carcinoma and negative findings at second-look laparotomy, eight (26.7%) had a recurrence of disease 2 to 63 months after the second look (median, 21 months). Seven patients had tumor recurrence in the abdomen or pelvis, and one patient, in the brain. Ninety-seven percent of patients with negative findings at second-look laparotomy were alive without evidence of disease after 1 year, and 67% remained alive and free of disease after 2 to 100 + months of follow-up (median, 24 + months). Five patients died 2 to 20 months after recurrence, and three continued on salvage chemotherapy. All but one of these eight patients had grade 2 or 3 tumors, and all but one had Stage III or IV disease. None of these 8 patients with recurrence after negative findings at second-look laparotomy had been treated with alkylating agent therapy.

Seven of the 14 patients with invasive carcinoma and microscopic positive findings at laparotomy were pro-

Table IV. Correlates of findings at second-loo_laparotomy (epithelial carcinomas)

	Negative	Microscopic positive	Grossly positive
Age (yr)			
≤40	4	5	3
>40	26	9	32
FIGO stage			-
I	3	1	1
II	6	Ô	ī
III	14	12	$2\hat{7}$
IV	6	1	4
Recurrent	ĺ	Ô	$\hat{2}$
Histologic grade	-	Ů	~
1	7 ·	5	7
2	12	6	18
3	ĪĪ	3	10
Cell type		Ü	10
Serous	12	10	23
Adenocarcinoma (not otherwise specified)	7	4	6
Endometrioid	7	Õ .	i
Mucinous	i	o o	3
Clear cell	3	å	2.
Largest residual (cm)	•	Ţ.	~
0	15	3	2
Ī	6	2	5
2	3	3	5
3	2	ī	3
4-6	0	2	3
>6	3	2	10
Unknown	1	· 1	7
Chemotherapy			
Single alkylating agent	8	3	8
Cisplatin combination	19	11	22
Other chemotherapy	3	. 0	4
No. of courses of chemotherapy	-	-	-
≤8	7	1	9
9-12	18	9	19
>12	5	4	6 .

gression-free 16 to 56+ months after the second look. Seven had progression, with a median progression-free interval of 14 months (mean, 21.7 months; range, 5 to 59 months). Six patients had tumor progressin in the abdominal cavity, and one patient had a bram metastasis. Seventy-nine percent of these patients were alive without evidence of disease 1 year after the secand look, and 50% remained alive and free of disease _iter 7 to 67+ months (median, 27.5+ months) of fallow-up. Intraperitoneal chromic phosphorus was a part of the salvage regimen in six of eight patients in the group with no progression but in only one of seven in the group of patients with disease that had przgressed. Only 11% of patients with gross disease at the time of the second look were alive and free of disea e after 2 to 50 months of follow-up (median, 18+ maths).

There was one intercurrent death (diabetes mellitus) and two toxicity-related deaths (one each of \(\)\cop oxorubicin cardiomyopathy and sepsis during sal-age chemotherapy). Favorable survival status for patents with invasive epithelial carcinoma correlated with findings at second-look laparotomy, FIGO stage, reschual disease at first laparotomy, and age but not with chemotherapy regimen or number of courses of \(\)\text{reatment}

(Table V). Survival status correlated with grade of tumor only for the patients with grade 0 disease, all of whom were alive and free of disease 19 to 41 + months (median, 29.5 months) after second-look laparotomy.

Comment

There has been a considerable evolution of thought since Wangensteen¹¹ first proposed secondary reentry of the abdomen in patients with carcinoma of the colon or rectum. He selected those patients with positive nodes at the initial operation for reexploration 3 to 4 months later in anticipation of "wiping up" clinically occult recurrent disease. Although he proposed multiple reentry procedures, he did not discuss an interrelationship between operation and other therapeutic modalities. Second-look laparotomy in patients with ovarian cancer has been most broadly used in teaching institutions as an end-staging procedure in order to document the comparative value of different treatment regimens. In this sense, the procedure has been more a part of chemotherapeutic research protocol than a part of the patient's therapy per se. The operation has been broadly applied in the management of ovarian cancer, however, and is widely considered to be a part

Table V. Correlates of survival status (epithelial carcinomas)

	Alive/no evidence of disease	Alive/disease	Dead/disease
Age (yr)			
≤40	7	0	5
>40	24	8	33
Second-look findings			
Negative	20	3	5
Microscopic positive	7	1	6
Grossly positive	4	4	27
FIGO stage			
I	3	0	2
ĪI	5	0	2
III	21	8	2 2 24
IV	$\frac{1}{2}$	0	7
Recurrent	Õ.	Ö	3
Histologic grade	Ü	ŭ	
I	10	2	6
2	9	5	. 22
3	12	Ĭ	10
Cell type	1.2	•	***
Serous	13	7	24
Adenocarcinoma (not otherwise specified)	9	ó	8
Endometrioid	5	ì	ì
Mucinous	ì	0	3
Clear cell	3	0	2
Largest residual (cm)	3	O	4
0	13	2	4
1	7	1	4
	3	2	6
2 3	3	I	0
=	<i>3</i> 2		2 2
4-6	2	1	
>6	3 0	1	11
Unknown	U	0	9
Chemotherapy	•		
Single alkylating agent	9	1	8
Cisplatin combination	21	6	24
Other chemotherapy	Ι	2	4
No. of courses of chemotherapy	· _	_	
≤8	7	3	6
9-12	20	3	22
>12	4	2	9

of the standard treatment plan rather than an investigational tool.

Like most authors,2 we chose to subject to exploration only those patients who were clinically free of disease. Other authors have elected to perform reexploration in patients with known residual disease in an effort to accomplish further debulking. Some researchers have reported that volume reduction at the second look results in enhanced survival,1 but they are contradicted by Raju et al.,3 who found no improvement in survival among those patients who had all macroscopic disease removed at the second look. The success of surgical debulking depends upon the availability of an active chemotherapeutic regimen. All of the patients in the series of Raju et al. received cisplatin, and 48 of 65 received combination therapy that included cisplatin. With 8 years' experience with cisplatin combination therapy, we found no active salvage chemotherapeutic regimen in patients in whom cisplatin failed. We would propose, therefore, that if second-look laparotomy has any impact on ultimate survival, it is exerted through the information it provides and consequent modifications in adjuvant therapies rather than through the operation itself.

The initial surgical stage and the volume of residual disease at the completion of the primary operation have been consistently associated with the findings at secondlook laparotomy. 1, 2, 5, 7 We found that 75% of patients with initial Stage I or II disease had negative secondlook findings compared to 31.3% of patients with initial Stage III or IV or recurrent disease. Similarly, 78.9% of patients with no residual disease had negative findings at second-look laparotomy, compared to 25.4% of patients with residual tumor at the completion of the first laparotomy. Most of the primary operations in our series were performed prior to referral by gynecologists or surgeons not specifically trained or experienced in gynecologic oncology. Even though the records of the patients were carefully reviewed at the time of referral and some patients underwent reexploration to confirm

the stage of disease, one may still assume that some of the patients with Stages I and II disease, that was thought to be completely resected, actually has Stage III disease and/or had residual disease. We relied upon the operative reports from the referring institution or spoke with the operating surgeon by phone in an attempt to document the largest tumor diameter at the completion of the primary operation. Despite these factors, there still remained nine patients for whom the volume of residual tumor was unknown. All af these patients were free of clinically measurable disease at the beginning of chemotherapy. As a group, however, they did poorly, with only one of nine having regative second-look findings.

A number of other characteristics have been associated with negative second-look laparotomy Endings. Berek et al.5 subjected their data to multivariate≡nalysis in an attempt to determine the relative significance of different variables. They excluded patients with sumors of low malignant potential and still found significant differences according to initial tumor grade. Even when we included our patients who were judged on review to have grade 0 disease, we did not icentify a correlation between grade and second-look Endings. In this respect, we agree with Raju et al.3 anc Cohen et al.7 in whose studies were large numbers of patients treated with cisplatin combination therapy. Our six patients with tumors of low malignant potential zere initially considered by our institution to have grade 1 tumors and were treated as patients with diseas∈ of comparable stage. There is no recognizable diffeence in terms of characteristics or outcome separating them from patients with tumors of higher grade except for short-term survival (median, 30.5 + months).

Age is another parameter that was found I-7 Berek et al. to be predictive of second-look laparotomy findings. In our series, however, 25% of patients under 40 years of age had negative laparotomy findings compared to 39% of patients over 40 years of age

Timing of the second-look laparotomy has lang been a question. Schwartz and Smith1 correlated the number of courses of therapy with negative findings a secondlook laparotomy as well as the likelihood of reurrence after a negative second-look procedure. The recommended that at least 12 courses of therapy be given before a second look is considered. By waring this obligatory 12 months, one can identify certain patients who are destined to have a progression of di-ease and spare them unnecessary reexploration. Twelv= months has become the "standard" timing for reexploration as a result of those authors' experience. There is an increased awareness of the risk of leukemia after longterm alkylating agent therapy.⁸ Broader use of combination therapy has resulted in a higher rate of objective response.6.7 Cumulative toxicity has precluded the administration of 12 cycles of cisplatin or doxorubicin. We initially proposed⁶ that cisplatin and doxorubicin be administered until tolerance dosages were achieved and that maintenance cyclophosphamide be given for a minimum total of 12 cycles before the second look. There is a trend toward earlier reexploration in patients treated with combination therapy.⁴

It can be pointed out that the most appropriate time to perform a diagnostic reexploration is when the therapy is changed and, conversely, the most appropriate time to change therapy is after diagnostic reexploration. Twenty of the patients in our series underwent reexploration early (eight courses), in most cases at the time of discontinuation of cisplatin and doxorubicin. We, like Berek et al.,⁵ discovered no correlation between number of courses of chemotherapy and findings at second-look laparotomy.

Most previous studies have been composed of patients treated primarily with alkylating agents.1.2 Two studies3, 7 included almost entirely patients who had been treated with cisplatin, alone or in combination. Our series (52/78), as well as that of Berek et al.5 (28/ 56), examined cisplatin-treated patients as well as patients who did not receive cisplatin. Berek et al. report that cisplatin therapy correlated positively with negative findings at laparotomy. We found no such correlation. There has been an obvious bias at both our institutions to use cisplatin therapy in patients with disease of more advanced stage and greater residual volume, as well as in patients with better performance status. If any meaningful comparison of treatment regimens is to be carried out, we would need to examine the total number of patients who received each regimen and know what percentage of patients became eligible for a second look. Different eligibility criteria for a second look from institution to institution further confound any attempt to compare treatment regimens. We do acknowledge, however, that cisplatin-based combination therapy is able to produce a significantly higher percentage of total responses and complete responses.

If one analyzes the value of second-look laparotomy in terms of findings at laparotomy, then one must be prepared to question the reliability of the findings. Any operation in which gross techniques are used while a search is made for evidence of microscopic disease is destined to have false negative findings. We found that 31% of patients (16/51) who had grossly negative findings had microscopic disease only. Since we did not routinely sample lymph nodes throughout this series, it is possible that occult disease in nodes escaped detection. However, none of our patients who had recurrence had isolated nodal recurrence. In the series of Berek et al., isolated nodal persistence at the second look was found in five of eight patients. We did encounter patients who had negative biopsy findings but

positive washings, unlike Schwartz and Smith.1 Recently, Phibbs et al.12 carefully analyzed the sites of persistent disease at second-look laparotomy and found that no patient had tumor at a new site when original sites were negative. This finding emphasizes the need for an accurate exploration and description of findings at the primary laparotomy as well as the need to sample all sites of known prior disease at the second-look laparotomy. In our series, if a positive biopsy finding was obtained on frozen section, then the procedure was terminated. Like Webb et al.,2 we found that peritoneal surfaces, particularly pelvic peritoneum, were the most common sites at which residual disease was seen.

The most consistent parameter predictive of survival has been the findings at second-look laparotomy. Like the patients of Gershenson et al.¹³ and Cohen et al.,⁷ our patients with negative findings at second-look laparotomy did well, with a 24+ month median survival and 67% of patients alive and free of disease from 12 to 100+ months after the second look. There are a significant number of recurrences after a negative second look, however.2.4.5.13 Some authors have proposed the continuation of chemotherapy after negative findings at laparotomy.2.3 Recurrence appears to be more likely among those patients who have negative findings at second-look laparotomy in the face of an advanced stage of disease or large residual volume2 and is predominantly intra-abdominal. Of our eight patients who had recurrences after negative second-look findings, six had received cisplatin combination therapy. This recurrence rate of 31.6% (6/19) is discouraging. Although cisplatin may be capable of inducing a large number of complete responses in patients with a large volume of residual disease, these responses may not be so durable as expected.

With further therapy after a second-look procedure, the patients with microscopic disease have a median survival rate of 27.5+ months, with 50% of patients alive and free of progression of disease at 14 to 67+ months of follow-up. Seven of these 14 patients had progression of disease from 5 to 59 months after second-look laparotomy. Like Copeland et al.,14 we found a good probability for extended survival in these patients. Only one of seven who received chromic phosphorus has had progression. We think that this form of adjuvant therapy offers benefits to patients who have microscopic persistent disease after cisplatin therapy. Webb et al.2 reported the use of phosphorus for patients who had positive findings at second-look laparotomy. Recently, Varia et al.15 reported their institution's experience with the use of chromic phosphorus, with only four recurrences among 12 patients with low-volume disease, and two recurrences among 26 patients with negative findings at second-look laparotomy.

If we intend to benefit our patients, we should ask

ourselves what information we wish to obtain at the second look, and what are the goals of this operation. Do we measure the efficacy of the operation by the percentage of negative findings at laparotomy, by the number of relapses after negative findings at laparotomy, or by the ultimate survival rate? Those patients who have negative findings at second-look laparotomy and those who have microscopic disease at the second look are those who benefit from a second look. Although most authors discontinue therapy if secondlook findings are negative, recurrence rates are sufficiently high in the abdominal cavity to consider a largescale prospective study of adjuvant phosphorus. Those patients with microscopic disease have a survival rate that closely approximates that of the patients with negative findings. Most of these patients have received continued chemotherapy in the past. Again, intraperitoneal phosphorus would appear to be a justified alternative in these patients.

If we propose to maximize the benefits of secondlook laparotomy, we should attempt to reduce the number of patients in whom gross disease is observed at laparotomy. Thorough clinical and radiographic evaluation to identify residual disease should be emphasized. We have been pleased with the reliability of computerized axial tomography in 41 patients in this series. There was only one false positive finding in this group, although false negative findings were more common. Others have reported their satisfaction with laparoscopy prior to laparotomy.16 Approximately one third of patients can be found to have gross disease by means of laparoscopy and thus can be spared laparotomy.

In the absence of any randomized studies, it is purely speculative as to how many patients have derived benefit from second-look laparotomy. Conceivably, if therapy had been continued or empirically discontinued in all patients without laparotomy, comparable results might have been achieved. It would appear that patients who originally had Stage I or II disease, which was accurately staged and completely resected, did not benefit from a second look. We would suggest that these patients should undergo reexploration only as a part of formal clinical trials to evaluate different treatment regimens. Other characteristics are not sufficiently reliable predictors to exclude patients from reexploration. As increasing numbers of patients receive cisplatin combination therapy and second-look laparotomy is performed in hospitals of all sizes, more questions will be raised about therapy after negative findings or findings of microscopic disease at the second look.

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Vitamin E concentrations in serum of newborn infants after topical use of vitamin E by nursing mothers

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Presented is a study that examined the effect of topi al application of vitamin E by nursing mothers. (AM J OBSTET GYNECOL 1985;152:668-70.)

Key words: Breast-feeding, vitamin E, newbo-n infant

Topical application of vitamin E in oil, expressed from capsules, has become a popular treatment for sore nipples early in the course of breast-feeding. Despite the absence of studies demonstrating vitamin_E's efficacy, major support groups for nursing mothers have recommended this drug, which is available without prescription in drug and food stores.

Adverse effects including sepsis and necrotizing enterocolitis have been described in premature newborn infants with elevated serum vitamin E levels after parenteral or oral administration of vitamin E.1 Although there have been no reported adverse effects of maternal topical vitamin E on nursing infants, we thought it important to examine the effect of this application of vitamin E oil on the serum vitamin E levels of newborn infants as an index of its safety.

We measured the increment in serum tocopherol concentrations from birth to day 6 of life in two groups of infants, those exposed to maternal topical vitamin E oil and those whose mothers did not use vitamin E.

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Methods

The research protocol was approved by the Committee for Protection of Human Subjects from Research Risks of Brigham and Women's Hospital, and informed consent was obtained from the parents of the infants.

We studied the healthy infants of 10 women who delivered at term and who had vitamin E capsules prescribed by their obstetricians for breast care. A control group was composed of 10 healthy term infants born to women who used no topical treatment (n = 5) or lanolin only (n = 5). Mothers in both groups were equally distributed between private and clinic services, and the two groups had similar distributions of age, race, and prenatal vitamin E exposure by history. Recruitment of both groups to the study took place during the same 6-month period. Group sizes were selected on the basis of power analysis to detect a true difference of 0.5 mg/dl or to rule out the same with a 85% certainty. All mothers received the standard hospital diet and no more than 30 IU of vitamin E in oral-supplement multiple vitamin preparations.

Babies nursed on demand, at least six times daily, and received no supplemental formula.

The use of vitamin E capsules (400 IU dl-α tocopheryl acetate, Ascot) by the mothers of study group infants was standardized as follows: (1) After each nursing, nipples were allowed to air dry. (2) A 400 IU capsule was opened with a sterile needle. (3) Contents of one capsule were applied to completely cover both areolae and nipples. (4) Oil was massaged into both areolae for 1 minute each. (5) Subjects dressed normally and nursed as usual at the next feeding. (6) No special attempt was made to wash or wipe off the oil from nipples, except during daily bath or shower, as permitted.

A 2.5 ml sample of blood was collected from the refrigerated, stored umbilical cord blood specimen. A second 2.5 ml sample of blood was collected by venipuncture from all infants at the beginning of the sixth day of life, immediately prior to nursing, and after approximately 21/2 days of maternal use of vitamin E oil, generally beginning on the third day after delivery.

Serum was harvested by centrifugation and stored at -20° C while protected from light until assay. Samples were analyzed by the spectrofluorometric method of Hansen and Warwick² at Biosciences Laboratories, Van Nuys, California.

Data were analyzed by Student's paired t test, by Wilcoxon signed rank test, and by replicated two-factor analysis of variance.

Results

The concentrations of vitamin E in umbilical cord sera from study and control infants were simi $lar (0.40 \pm 0.14 \text{ versus } 0.34 \pm 0.12 \text{ mg/dl}, p > 0.05,$

mean \pm SD). In contrast, by day 6 the serum vitamin E concentration in the group of infants exposed to maternal topical vitamin E was significantly greater than that of control infants (1.75 \pm 0.57 mg/dl versus 1.22 ± 0.37 mg/dl, p < 0.025). The percent increment from cord levels in serum vitamin E concentrations was also significantly greater in vitamin E-exposed infants $(361.7 \pm 122.3 \text{ versus } 274 \pm 98.5 \text{ percent}, p < 0.05).$ The time of sampling was similar for the two groups $(124 \pm 16.9 \text{ versus } 118.75 \pm 6.9 \text{ hours}).$

No newborn infant in either group had clinically appreciable hepatosplenomegaly on physical examination on day 1 or day 6, and no clinical evidence of adverse effects related to maternal use of vitamin E oil was observed for either mother or child.

Comment

Sore nipples are a common complication of breastfeeding occurring in approximately 60% of women when nursing is initiated. Although proper positioning of the nursling at breast is the key to both preventing and treating nipple pain, many proprietary treatments have been used routinely for this problem (lanolin, Masse breast cream, Vaseline). At Brigham and Women's Hospital approximately 20% of nursing mothers have vitamin E capsule contents prescribed for topical nipple care. This treatment is prescribed by individual obstetricians, based on anecdotal experience with its efficacy and on strong support by the nursing staff. As with any treatment involving drugs in the perinatal period the issue of safety for the newborn infant has heen raised

Newborn infants at term demonstrate lower umbilical cord serum vitamin E concentration than their mothers even with prenatal supplementation. Cord blood samples from all of the infants in this study were similar to those previously reported for term infants. When infants are breast-fed after birth, serum vitamin E levels rise to the normal adult range (1.0 to 3.1 mg/ dl) within 6 days, and this expected increase was seen in both the control and the study infants. However, serum vitamin E levels measured on day 6 in newborn infants exposed to maternal topical vitamin E were significantly greater than in the control infants. The highest observed vitamin E concentration was 3.1 mg/dl, occurring in an infant in the study group; no differentiating factor could be identified in this case (such as longer or more frequent use of vitamin E, or unusual

Newborn infants were potentially exposed to very large amounts of vitamin E. The recommended dose for supplementation of vitamin E for the newborn infant is 5 IU, or milligrams, daily.3 Although we did not attempt to quantify the actual vitamin E intake of study infants, the nursing infant is potentially exposed to as

much as 3200 IU daily (400 IU capsules applied Etimes per day). This would constitute approximately 1000 U/kg/day for the average infant. Although much of this may be rubbed off onto clothing, it is apparent from the higher serum vitamin E levels in the exposed infants that either some vitamin E is directly ingested and absorbed by the nursing infant or there it an elevated milk content of vitamin E caused by maternal systemic absorption. Either mechanism or both may be important.

In summary, the brief use of the contents of =C0 IU capsules of dl- α tocopheryl acetate by their mo hers as a nipple balm resulted in higher serum vitamin E levels in term infants. Although serum vitamin E levels in term infants. Although serum vitamin E levels es remained within the normal range on day 6, the study demonstrates that even a brief topical use of viarnin E by the nursing mother can result in increased situmin E levels in the infant's serum. The consequence of total body vitamin E stores remains uncertain.

Although the risk of serum vitamin E concentration elevated to this degree may be small, excessive or prolonged use of this agent may result in potentially harmful effects for the newborn infant. As there is no objective evidence for efficacy of vitamin E treatment in the prevention of sore nipples, we would recommend proper study of this treatment so that benefits as well as risks can be objectively assessed. Meanwhile, if mothers continue to use this therapy, physicians should strive to limit the amount and duration of vitamin E use to the first several days of nursing.

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Goiter in pregnant teenagers

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Enlargement of the thyroid gland is common in adolecent girls, but there have been no previous reports of the frequency of goiter in pregnant teenagers. This study was undertaken to determine the prevalence of goiter in 309 consecutive pregnant adolescent patens, ages 11 to 19 years, who presented for prenatal care at the University of California (San Diego) Medical Center Teer. OB Clinic. Eighteen patients (6%) were found to have a goiter. Subsequent diagnostic ess revealed that 28% of the patients had autoimmune thyroid disorders (chronic thyroiditis or Graves' disease), and the remainder had nontoxic goiters or subacute thyroiditis. Black patients had significantly more thyroid disease than Mexican-American (p < 0.01) or white patients (p < 0.005). The incidence of thyroid problems during pregnancy did not differ significantly from that of 600 nonpregnant teeragers who came to the general Adolescent Medicine Clinic for other health problems. Because entrymalities in thyroid function may have potentially adverse implications for mothers and their infants, we recommend careful evaluation of all pregnant teenagers with goiter to assess maternal thyroid function and to provide treatment if necessary. (AM J OBSTET GYNECOL 1985;152:670-4.)

Key words: Thyroid, goiter, teenage pregnanty

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Reprint requests: Dr. Thomas J. Long, Department of ⇒ediatrics, Adolescent Medicine Division (H-814-F), UCSD Medi-al Center, 225 West Dickinson St., San Diego, CA 92013. Erlargement of the thyroid gland is commonly observed in adolescents, with a high female-to-male sex distribution. Estimates of the frequency of goiter in this age group range from 2% to 8% in North America, where geographic areas of iodine deficiency no longer exist.¹⁻⁷ The possible role of pregnancy as a cause of goiter is controversial. Although the authors of older reports linked thyroid gland enlargement with normal pregnancy,³⁻¹⁰ other investigators have ques-

tioned this view in areas in which iodine intake is adequate.11-12

Reports of the prevalence of goiter in pregnant women of all ages are sparse. Murray⁵ noted that 4.9% of pregnant women in a Canadian study had goiters. An enlarged thyroid gland has been reported as an incidental finding in 0.9% of adolescent girls during their first pregnancy,7 but there have been no previous reports of a systematic prospective examination of the thyroid gland in pregnant teenagers.

The present study was undertaken to assess the prevalence of enlargement of the thyroid gland in pregnant teenagers attending a multiracial prenatal clinic.

Material and methods

Subjects. The study population included 309 consecutive pregnant adolescent girls who were admitted to the University of California, San Diego Medical Center Teen OB Clinic from August, 1978, through December, 1982. The ethnic composition of the population studied was Mexican-American (35%), white (34%), black (25%), and others (6%). The mean age of the group was $16.4 \pm SD$ 1.3 years. Most girls were from a low-socioeconomic background.

We also reviewed the hospital records of 600 adolescent girls who presented to the Adolescent Medicine Clinic from June, 1979, through August, 1983, to establish the prevalence of goiter in nonpregnant adolescents. Careful examination of the thyroid gland was routinely performed in the Adolescent Medicine Clinic, and each patient was checked by an attending faculty member who was also involved in the evaluation of our pregnant teenagers. The mean age of the comparison group was 15.5 ± SD 2.0 years. There was no significant difference in the racial compositions of the pregnant and nonpregnant groups.

Procedures. At the first prenatal visit, all pregnant teenagers were given a complete physical examination by a pediatric or obstetric resident. The mean gestational age for the first visit was 22 weeks. All patients with an enlarged thyroid gland or signs or symptoms of thyroid disease had the condition confirmed by a second examiner. An enlarged thyroid gland was defined as visible and/or palpable and having a transverse span of ≥6 cm. In all instances in which the transverse span of the thyroid gland exceeded 6 cm, the gland was readily visible with the neck slightly extended (therefore, at least a World Health Organization grade 1B goiter4).

All pregnant patients with goiter were evaluated for clinical signs or symptoms of thyroid dysfunction. Tests of thyroid function (serum thyroxine, thyrotropin, thyroid autoantibody titers, and calculation of the free thyroxine index) were carried out at first observation and were repeated either when indicated clinically or at least during each trimester. Radioactive iodine uptakes and scans were not used because of possible risk to the fetus. Fetal well-being was assessed throughout pregnancy by estimation of intrauterine growth, fetal heart rate, and other biophysical tests whenever necessary. Thyroid disorders were classified by clinical and laboratory criteria.

Nontoxic goiter. Thyroid gland enlargement was not associated with thyrotoxicosis or hypothyroidism and did not result from an inflammatory or neoplastic process. These thyroid glands were soft, smooth, nontender, and diffusely enlarged.

Subacute thyroiditis. A tender and enlarged thyroid gland was noted after a viral illness. Pain in some individuals radiated to the neck or postauricular area. Elevated free thyroxine indices and mild transient symptoms of hyperthyroidism were common early in the clinical course.

Hashimoto's thyroiditis. A diffuse sometimes asymmetric thyroid gland with firm to hard consistency was noted. The goiter was associated with positive titers of serum antithyroglobulin or antimicrosomal antibodies. Metabolic status and thyroid function studies varied with stage of the disease.

Graves' disease. There was a diffuse goiter and exophthalmos in the presence of clinical and laboratory evidence of thyrotoxicosis.

Laboratory tests. Measurements of serum thyroxine were performed with use of the Corning solid-phase radioimmunoassay kit. Serum triiodothyronine was measured by the Mallinckrodt coated-tube radioimmunoassay method. A Beckman solid-phase human thyrotropin kit was used to measure serum levels of thyrotropin. The free thyroxine index was calculated as the product of the total thyroxine and the triiodothyronine uptake divided by a standard. Antithyroglobulin antibody titers were measured by an indirect hemagglutination test with use of a Thymune-T kit purchased from Wellcome (Beckenham, England), and antimicrosomal antibodies were measured by the Wellcome Thymune-M hemagglutination kit.

The data were analyzed by χ^2 analysis.

Results

Table I shows the racial distribution, age, and prevalence and diagnosis of thyroid disorders in the 309 consecutive pregnant teenagers with goiter. Eighteen pregnant teenagers (6%) had a goiter, and black pregnant teenagers had a significantly higher percentage of goiter (14%) than did Mexican-Americans (4%, p < 0.01) or whites (2%, p < 0.005). Most thyroid gland enlargements (89%) were detected at the initial clinic visit at a mean gestational age of 22 weeks and ranged

Table I. Racial distribution, age, and prevalence and types of thyroid disorders in pregnant and nonpregnant adolescent girls with goiter

. Race	Total patients		Goiter		Nontoxic	Hashimoto's		CL
	n	Mean age*	n	%	goiter	thyroiditis	Graves' disease	Subacute thyroiditis
Pregnant (N = 309)		,	•					
Mexican-American	108	16.6	4	4	3	_		1
White	106	16.2	2	2	1 '		1	_
Black	77	16.4	11	14†	4	3	1	3
Other	18	16.3	1	6	1	_	_	
Total ·	309	16.4	18	6	9	3	2	4
Nonpregnant (N = 600))							
Mexican-American	141	15.9	5	4	2	1.	1	1
White	289	15.9	13	5	9	4		
Black	137	15.4	. 5	4	4	1	_	
Other	33	15.8	4	12	3	1		
Total	600	15.5	27	. 5	18	7	1	1

^{*}No significant difference between pregnant and nonpregnant women.

Table II. Mean values for laboratory tests in pregnant teenagers with goiter

Test	$Nontoxic \ goiter \ (n = 9)$	Subacute thyroidits (n = 4)	Hashimoto's thyroiditis (n = 3)	Graves' disease (n = 2*)
Free thyroxine index	7.6	14.2	21.2	14.2
Total thyroxine	12.8	14.8	19.5	21.2
Triiodothyronine radio- immunoassay	213	185	NA	NA
Thyrotropin	5.1	. 4.E	2.2	3.5

Numbers in parentheses indicate number of patients in each group. MA, Not available.

in transverse span from 6.0 to 11.0 cm. No solitary nodules were observed.

Table II describes the mean values for thyroid function tests obtained at the time of discovery of the goiter. Appropriate clinical evaluations and laboratory tests performed on the pregnant adolescents revealed that 28% had autoimmune thyroid disease, and 22% had subacute thyroiditis. Four patients had subacute thyroiditis, Hashimoto's thyroiditis was present in three patients, and two girls had Graves' disease. The remainder of the patients with thyroid gland enlargement (50%) had a simple nontoxic goiter.

The clinical course of the four patients with subacute thyroiditis was as follows. Two were euthyroid at diagnosis and remained so, one was initially hypermetabolic and became euthyroid within 4 weeks, and the fourth had a mildly elevated free thyroxine index and tender gland until delivery. At 2 weeks post partum, she had normal laboratory values and a nontender goiter, which was no longer palpable by 6 weeks post partum.

None of the teenagers with *nontoxic* goiters became hyperthyroid or hypothyroid during pregnancy. However, four patients (22% of those with goiter) were hy-

perthyroid during pregnancy, and two required treatment. One patient with Graves' disease (at 25 weeks' gestation) and another with Hashimoto's thyroiditis (at 28 weeks' gestation) were hypermetabolic, with elevated free thyroxine indices. Both received treatment with propylthiouracil and propranolol. Two additional hyperthyroid patients did not receive therapy. One of them had Graves' disease and the other had subacute thyroiditis, but both were only mildly hypermetabolic and were not treated.

All three patients with Hashimoto's thyroiditis had elevated antithyroid antibody titers (microsomal fraction >1:10 in all three; thyroglobulin fraction >1:5120 in one patient). Serial determinations were not obtained.

Fifteen of 16 infants of mothers with enlargement of the thyroid gland were normal at birth, including those infants whose mothers received treatment for hyperthyroidism. The baby of the mother with subacute thyroiditis and hyperthyroidism was born prematurely at 35 weeks' gestation. He was euthyroid and normal on examination yet was small for gestational age, with a birth weight of 1500 gm. The placenta was atrophic and weighed 200 gm. Plasma cell infiltration was noted,

[†]Black versus white, p < 0.005; black versus Mexican-American, p < 0.01 by χ^2 analysis.

^{*}One patient received propylthiouracil treatment.

but cultures and serologic tests for infection from both the infant and the placenta were negative. We did not observe postpartum autoimmune thyroiditis with either transient hypothyroidism or hyperthyroidism, as reported by others,13 in any of our teenage mothers with goiter. All patients were examined at 6 weeks post partum whenever possible, and the hyperthyroid teenagers were followed up for 6 months.

In the comparison group of 600 nonpregnant girls who were examined in the University of California (San Diego) Adolescent Medicine Clinic during the same 4year period, 27 (5%) patients were noted to have goiters (Table I). All thyroid glands were diffusely enlarged and no nodules were detected. Nontoxic goiter was the most common diagnosis (66%), with autoimmune thyroiditis (30%) second in frequency. All of the nonpregnant teenagers with goiter were clinically euthyroid. The prevalence of Hashimoto's thyroiditis in the nonpregnant teenagers was 1%, which was identical to that in the pregnant adolescents.

Comment

No previous reports from obstetric clinics for teenagers have assessed the frequency of enlargement of the thyroid gland during pregnancy in this age group. Although enlargement of the thyroid gland was once thought to be a characteristic feature of pregnancy,8-10 more recent reports have failed to support this view in geographic areas in which iodine deficiency does not exist.11, 12 The present study failed to demonstrate a higher frequency of goiter in pregnant than in nonpregnant teenagers, which indicates that abnormalities of size and function of the thyroid gland are not more prevalent during the stress of reproduction at a young age.

The finding of enlargement of the thyroid gland in 6% of pregnant teenagers suggests that the necks of all young pregnant women should be carefully examined. When a goiter is detected, an assessment of maternal metabolic status is indicated. A simple evaluation can be incorporated into the first prenatal examination. In most instances, the history, physical examination, and appropriate laboratory studies (serum thyroxine, thyrotropin, a calculation of the free thyroxine index, and measurement of antithyroid antibodies) usually enable the physician to make an accurate clinical diagnosis. Diagnostic studies with radioactive iodine are contraindicated during pregnancy. Ultrasonographic scans of the thyroid gland during pregnancy are indicated in the presence of an enlarging solitary nodule.

In many hyperthyroid patients it is difficult to distinguish Graves' disease from the hypermetabolic stage of Hashimoto's thyroiditis ("Hashitoxicosis"). Without microscopic examination of the gland, the latter condition is a clinical judgment based on the finding of a firm rather than soft goiter. At present, the current view of some thyroidologists is that these two autoimmune disorders overlap and occur in individuals with specific HLA tissue types (DR3, DR4), and GM allotypes. In our patient, we thought the clinical examination, lack of exophthalmos, and long clinical course were more consistent with Hashimoto's disease.

Although only one infant in our study was born prematurely with intrauterine growth retardation, the implications of maternal thyroid disease for the fetus and newborn baby are significant. Previous reports of maternal hypothyroidism14, 15 indicate an increased risk of spontaneous abortion, prolonged gestation, and stillbirth. Other investigators 16 have implied that maternal hypothyroidism is associated with subsequent impairment of intellectual development in the child. Maternal Graves' disease increases the likelihood of spontaneous abortion, intrauterine death, or growth retardation and prematurity.17 Infants of these mothers are also at risk for neonatal Graves' disease and developmental problems. 18 In addition, the pharmacologic treatment of maternal hyperthyroidism may adversely affect subsequent growth and development of the infant.18, 19

Maternal autoimmune thyroid disorders may cause postpartum fluctuations of thyroid function. 13, 20 These changes may be expressed by either transient hyperthyroidism or hypothyroidism. Because goiter and autoimmune thyroid disorders are common in women of all ages, the courses of these patients should be monitored carefully during gestation and the first 6 months post partum. Infants of mothers with Graves' disease and other autoimmune thyroid disorders should be examined at regular intervals during early childhood to monitor growth and development.19

In conclusion, goiter and other thyroid disorders were observed in 6% of pregnant teenagers, but this frequency did not differ from that in nonpregnant adolescent girls. Pregnant black women had a significantly higher prevalence of thyroid gland enlargement than Mexican-American or white women, but the reason for this is not apparent. The diagnosis of goiter in pregnant teenagers was most often simple nontoxic goiter (50%), but autoimmune thyroid disease (28%) and subacute thyroiditis (22%) were also noted.

We wish to express our special thanks to Mr. Paul Shragg for statistical analysis of the data.

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Laser treatment of cervical intraepithelial neoplasia in an office setting

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One hundred eighty-six patients underwent carbon dioxide laser treatment of cervical intraepithelial neoplasia. Both vaporization and excisional procectres were performed in an office setting without difficulty. Thirty-nine patients (36.4%) had grade 1_E38 (35.6%) had grade 2, and 30 (28%) had grade 3. Among 107 patients followed up for at least 6 morths, there were two treatment failures (5.1%) in the grade 1 group and no treatment failures for grades 2 and 3. The overall success rate for all grades of cervical intraepithelial neoplasia was 98% for a sirtle laser treatment. Our ability to use the laser to excise a specimen, as well as to treat large and endocertal lesions, allowed the office treatment of many patients who would otherwise have required hospitalization. (AM J OBSTET GYNECOL 1985;152:674-6.)

Key words: Cervical intraepithelial neoplas≡, laser, office surgery, conization

Methods for treating cervical intraepithe al neoplasia can be classified as either those that extroy the lesion or those that excise the lesion. Destrutive methods include cryosurgery, radical diathermy and laser vaporization. With these methods, one must positively rule out invasive cancer by cytologic examination, col-

poscopy, directed biopsy, and endocervical curettage. When invasive cancer is not absolutely ruled out, one must resort to excisional methods, such as conization, that require an operating room setting and general anesthesia.

For many years, cryosurgery was the only treatment method adaptable to a physician's office. Treatment failure rates with cryosurgery appear to depend both on the grade of cervical intraepithelial neoplasia and on the size of the lesion. Townsend' showed that there was a failure rate of 7% with small lesions, whereas the overall failure rate was 42% with large lesions, irrespective of grade. Creasman et al.² found that grade was a more significant factor, with a 17.7% failure rate

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Table I. Cases followed 6 months or longer

Cervical intraepithelial	Treated per group		Persistent disease		
neoplasia .	No. ·	. %	No.	%	
I	39	36.4	2	5.1	
II	38	35.6	. 0	0.0	
III	30	28.0	0	0.0	
Total	107	•	2		

for one treatment for grade 3 cervical intraepithelial neoplasia.

Contraindications to cryosurgery include a positive endocervical curettage, discrepancy between cytologic and biopsy findings, or colposcopic findings suggestive of invasion. Endocervical extension of cervical intraepithelial neoplasia is considered to be a relative contraindication to freezing. When one excludes women with these contraindications, many are not candidates for cryosurgery. If one chooses not to freeze grade 3 cervical intraepithelial neoplasia and/or large lesions, an even larger group of women is excluded. These women usually undergo cold conization or, less often, hysterectomy. Considerable savings might be expected if these women could be treated in the physician's office.

Recently, there has been much interest in the use of the carbon dioxide laser as a method of treatment for cervical intraepithelial neoplasia. With a knowledge of the location of the transformation zone and the lesion, one can plan an appropriate procedure to eliminate the lesion. Anderson and Hartley³ showed that, 99.7% of the time, involvement of cervical crypts with cervical intraepithelial neoplasia is <3.8 mm. The deepest involved gland in their series was 5.22 mm. Not surprisingly, the success rate of treatment is directly related to the depth of treatment.⁴ In practice, when the transformation zone is eradicated to a consistent depth of 6 to 7 mm, a failure rate of <5% can be expected with a single treatment.⁵

Although laser vaporization procedures often are performed in an office or clinic setting, excisional procedures are still traditionally performed in an operating room. This report summarizes the our experience in performing both vaporization and excisional procedures in an office setting:

Material and methods

One hundred eighty-six patients were treated with the carbon dioxide laser between December, 1982, and March, 1984. One hundred fifty-seven patients underwent vaporization procedures, and 29 underwent either excisional procedures or a combination of excisional and vaporization procedures. The size of the lesions varied from a few millimeters to extensive lesions that covered the cervix and extended onto the

vagina. No patients were excluded from laser treatment because of the size or grade of the lesion or the desire for sterilization. All patients had colposcopy and directed biopsy; most patients also had an endocervical curettage. All colposcopy and treatment was carried out by Dr. Indman, and all pathologic specimens were read or reviewed by Dr. Arndt. If the upper extent of the transformation zone was not positively identified by conventional colposcopy, the cervix was stained with toluidine blue and the cervical canal was examined with the Hamou microcolpohysteroscope. Thus the upper extent of cervical involvement of intraepithelial neoplasia could be identified in all cases, and an appropriate procedure planned.

If the standard criteria for ablative treatment were met, a Merrimack 810 carbon dioxide laser was used at power densities of 650 to 1200 W/cm² to evaporate a cylinder of tissue at least 7 mm deep into the cervical stroma and often significantly deeper if there was endocervical involvement. Paracervical block anesthesia made the procedure painless.

Situations that required a specimen included unsatisfactory colposcopy, positive endocervical curettage, discrepancy between cytologic and colposcopic or biopsy findings, or colposcopic findings suggestive of microinvasion. After we had performed a number of vaporization procedures, it became obvious that the effect on the cervix was the same whether the tissue was vaporized or excised. The logical extension of doing vaporization procedures in the office is to use the laser to do excisional procedures in an office setting. The technique of using the laser to perform a cone biopsy in the office has been described.7 By narrowing the laser beam to 1.0 to 1.5 mm, it was possible to use the laser to excise a cylindrical specimen of cervix. A satisfactory specimen for pathologic examination was obtained in all cases, and the loss of blood was usually less than that produced by routine cervical biopsy.

A combination procedure that consisted of vaporization of the ectocervix and excision of a central cylinder was used also when a large ectocervical lesion extended up the endocervix.³

Results

One hundred seven of the 186 patients treated have been followed up from 6 to 12 months, have had at least two follow-up examinations that consisted of cytologic and colposcopic evaluation, and are included in follow-up data. The results of treatment are shown in Table I.

Two patients (1.9%) were identified as having residual or recurrent disease. One patient treated for grade I cervical intraepithelial neoplasia had cytologic and biopsy evidence of moderate dysplasia with warty changes at her 3-month follow-up. She was re-treated and was free of disease at her next follow-up. The second patient with persistent disease after treatment for grade I cervical intraepithelial neoplasia also had recurrent vulvar warts. Biopsy after 12 months showed moderate dysplasia with condylomatous features. Both failures appear to have been caused by reinfection by papillomavirus rather than inadequate removal of tissue.

An additional patient had an excisional laser procedure. Histologic evaluation showed grade 3 cervical intraepithelial neoplasia with clear surgical margins. At 3 months, she had normal colposcopic findings, but cytologic and biopsy study showed grade 1 cervical intraepithelial neoplasia with koilocytosis. She was not retreated and at 6 and 12 months had normal cytologic and colposcopic findings and a biopsy specimen that showed reactive changes. Her case is currently not being classified as a treatment failure, since we think that the epithelium more likely represents repair than cervical intraepithelial neoplasia.

Six patients had delayed bleeding that required treatment, which was accomplished on an outpatient basis in all cases. Most instances of delayed bleeding occurred early in the series.

Comment

It should be emphasized that these patients will need to be followed up for many years before the final results are known. Nevertheless, preliminary results with the carbon dioxide laser show an extremely low rate of failure, especially for the lesions of higher grade. Since the end result of laser treatment is the removal of tissue, one has no reason to expect failure rates any higher than those with cold conization. Equally important is that, unlike the outcome with many treatment modal-

ities, healing after laser treatment usually leaves the squamocolumnar junction in a position easily accessible to cytologic and colposcopic study.

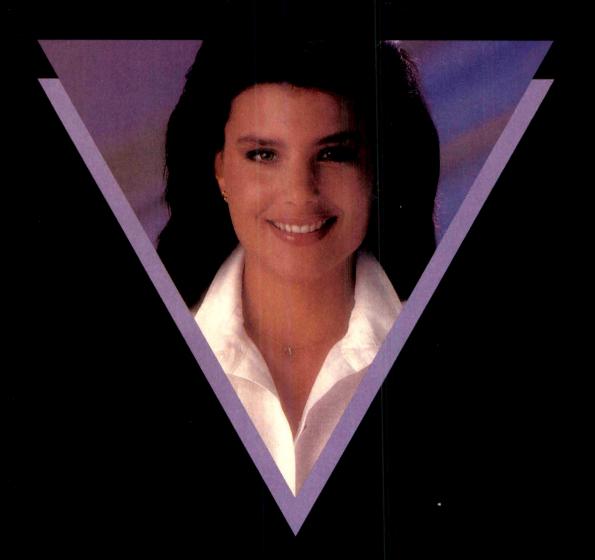
There is a growing insistence in the United States for the treatment of patients on an outpatient basis. We have seen too many cases of the use of cryotherapy in which the initial physician could not see the entire transformation zone with the colposcope or in which there were dysplastic fragments in the endocervical curettage specimen. Since the laser allows one to obtain a specimen of cervix on a par with that obtained by cold conization, one may now treat these patients in the office. The cost savings for doing an excisional procedure in the office is substantial. It is impressive to see a patient get up and walk away minutes after a large excisional procedure. Many patients in this series had lesions that would have precluded office treatment before the laser was introduced.

In summary, the carbon dioxide laser is an effective tool for the treatment of cervical intraepithelial neoplasia. Low rates of failure, as well as the physician's ability to perform both vaporization and excisional procedures in an office setting, may make the carbon dioxide laser the treatment of choice for cervical intraepithelial neoplasia.

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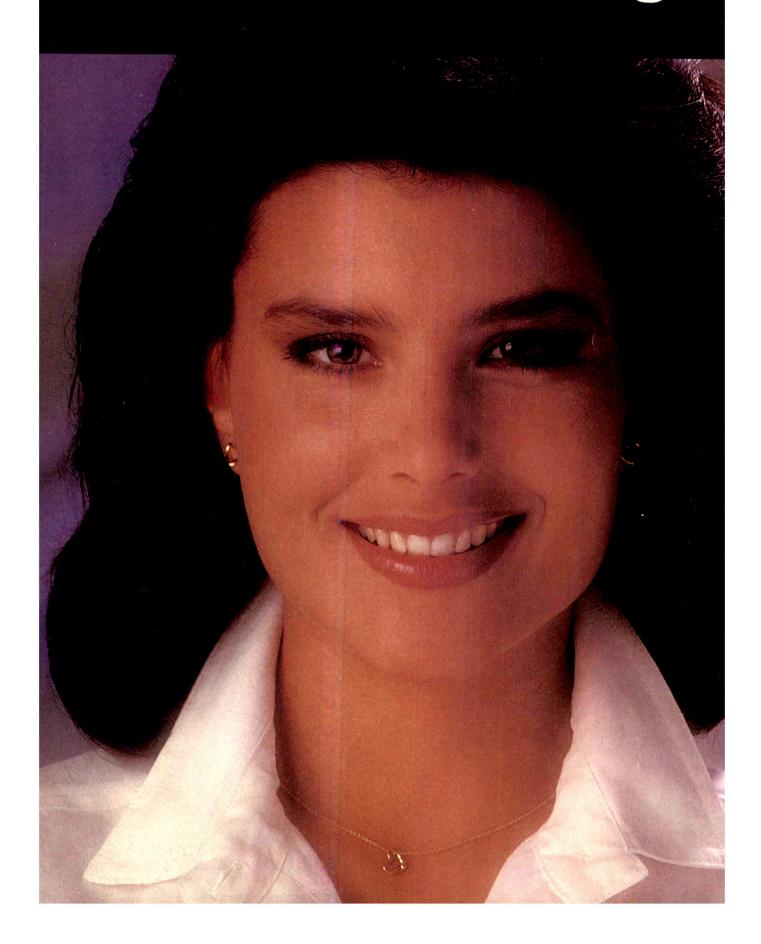
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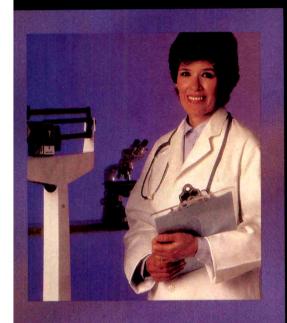
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To sneed.

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Practicioners prescribung Ous should be leatined when the londering innovation feating to these insks.

1. Thromboembolic Disorders and Other Vascular Problems. An increased risk of thromboembolic and thrombole disease associated with OC use is stabilished one study demonstrated an increased relative risk for latal venous thromboembolism and several studies demonstrated if for non-latal venous thromboembolism. They estimate that OC users are 4-11 times more likely than nonusers to develop these diseases without evident cause. One British study propried an excess death rate of 40% in OC users, most of which resulted from cardiovascular disease. Another British study showed a lower death rate in OC users than controls; an increase in cardiovascular disease has a located from cardiovascular disorders in Cardiovascular disorders in controls; an increase of mortality rates or more activated as the control of the

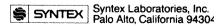
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MYOCARDAL INFARCTION (MI). Increased relative risk of MI associated with OC use has been reported. One British study found that the greater the number of underlying risk factors for cornary artery disease (cigarette smoking, hypertension, hypertensio

CONDITIONS—RISK ASSOCIATED WITH USE OF OCS				
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Heavy smokers	C	В	A	
Light smokers	D	C	8	
Nonsmokers				
(no predisposing conditions)	D	C.D	С	
Nonsmokers				
(other predisposing conditions)	С	C.B	B.A	

Physician and satent should be alert to earliest manifestations of thromboembolic and thrombotic daordras (e.g., thrombophields, pulmonary embotism, certorascular immediations) consistency control colocutions, colocutions, and immediately. A4-6 fold increased risk of gost surgery thromboembolic complications has been propried in OC users It lesable, discontrine OCs at least 4 weeks before surgery associated with norseard risk of thromboembolism or prologodal membrations and the propried in OC users It lesable, discontrine OCs at least 4 weeks before surgery associated with norseard risk of thromboembolism or prologodal membrations of the leg. The risk depending on the seventy of the variousties. 2. Occur Lesaons Neutro-ocular easions such as optic neutrits or retinal thromboes have been associated with OG user Discontrine OCs if there is unexplained, sudden, or parallederia, or retinal viscular lesaors, and institute appropriate diagnostic and herapeutic measures. 3. Carcinome Long-term continuous administration of natural or synthetic estropen in certain animals increases certain tumors, being in religional transplant, and the prologodal control studies presented animal control studies. The control studies provided the control studies provided the control studies provided the control studies provided the control studies. Design and malignant, in dogs. Several retiospective case-control studies presented an increased risks for this 1-39 times is sociation and control studies provided the control studies and control studies and control studies. The control studies are studies and control studies and control studies and control studies. The control studies are studies and control studies and control studies and control studies. The control studies are control studies and control studies and control studies and control studies. The control studies are control studies and control studies and control studies and control studies. The control studies are control studies and control studies and control studies and cont

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Conservative management of small bowel obstruction

B. Frederick Helmkamp, M.D., and Jay Kimmel, B.S.

Falls Church, Virginia

Intestinal Cantor tubes were used in the management of 69 gynecologic patients with pelvic malignancies who presented with small bowel obstruction. Small bowel obstruction was secondary to radiation injury, persistent or recurrent carcinoma, or postoperative adhesions. Thirty-one patients (45%) in this series had successful resolution of their small bowel obstruction with a Cantor tube, including 12 of 14 patients (86%) with postoperative adhesions. Complete obstruction of the small bowel was the only prognostic factor definitely associated with tube failure. Seventy percent of all patients had successful passage of the tube on one attempt, and no major complications were encountered. The Cantor tube has proved to be safe, effective, and easy to use, and guidelines for the management of it are included. Cantor tube decompression should be considered in the initial management of small bowel obstruction, since a significant percentage of the patients with this condition will not require surgical intervention. (AM J OBSTET GYNECOL 1985;152:677-9.)

Key words: Small bowel obstruction, Cantor tube, conservative management

Postoperative adhesions account for most cases of small bowel obstruction. In two large series, ^{1, 2} gynecologic operations preceded small bowel obstruction in 25% to 35% of cases. Intestinal obstruction may also be due to radiation therapy injury⁸ or advanced pelvic malignancies. ^{4,6} Therefore, small bowel obstruction, although infrequently encountered, may present a formidable challenge to the obstetrician-gynecologist.

The management of patients with small bowel obstruction remains controversial. Some authors recommend immediate surgical intervention, whereas others suggest conservative therapy with a trial of long tube decompression. In this article, we report our experience with the Cantor tube in the treatment of small bowel obstruction.

Patients and methods

Sixty-nine patients with a history of pelvic malignancy who presented with clinical and radiographic evidence of small bowel obstruction at Jackson Memorial Hospital (University of Miami) and Strong Memorial Hospital (University of Rochester) were studied prospectively by us between July, 1978, and December, 1983. Data were analyzed for etiology of the small bowel obstruction, success or failure of the Cantor tube, and complications associated with its use.

Patients with small bowel obstruction commonly presented with nausea, vomiting, abdominal distention, and pain. The clinical impression of small bowel obstruction was confirmed by supine, erect, and left lateral decubitus roentgenograms of the abdomen which showed distended, fluid-filled loops of bowel proximal to the obstruction. In six patients (8.7%), contrast studies through or around the Cantor tube confirmed complete obstruction of the small bowel.

Cantor tube management. With the patient placed in an upright position, the Cantor tube is passed through one nostril and into the oral pharynx. The tube is pulled out through the mouth, and 5 to 9 ml of mercury is injected into the middle of the bag with a No. 21 gauge needle. The mercury-filled bag is then gently placed into the oral pharynx and swallowed. We prefer this technique of insertion to passage of the mercury-filled bag through the nose, since the patient's compliance is much greater. The tube is attached to low intermittent suction and left freely mobile, since fixation to the nose will preclude further advancement.

The Cantor tube is left in place until there has been clinical and radiographic resolution of the small bowel obstruction. The following guidelines are then used. Day 1: Tube suction is changed to gravity drainage for 24 hours. Day 2: The tube is clamped for 24 hours to ascertain whether the patient can handle the succus entericus. Day 3: Liquids are allowed around the tube. Day 4: The tube is slowly removed. When the mercury-filled bag reaches the oral pharynx, it is withdrawn through the mouth, and the distal end of the tube with the bag is cut. The remainder of the tube is pulled through the nose.

Results

Obstruction associated with radiation injury, Radiation therapy was the main cause of small bowel obstruction in 33 patients (48%). Cervical carcinoma ac-

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Table I. Etiology of small bowel obstruction

Primary factor	No. of patients	T.
Radiation injury	33	48
Malignancy	22	3 2
Adhesions	14	20
	69	100

counted for 22 (67%) of these cases. Twenty patients had had an operation prior to radiation therapy. Twenty-nine patients had pelvic radiation therapy, two patients had total abdominal radiation therapy, and two patients developed small bowel obstruction during a course of pelvic radiation therapy. Four of these 33 patients were found to have complete obstruction of the small bowel, and all four subsequently required an operation. The mean duration of tube drainage in these 33 patients was 10.5 days (range, 1 to 33 days).

The Cantor tube restored bowel function for at least 30 days in 12 patients (36%). Of these 12 patients, eight had no further bowel problems, two required laparotomies for a second episode of small bowel obstruction (at 5 weeks and at 6 months), and two required a second Cantor tube (at 7 and at 12 weeks) and then had no further complications. Nineteen patients (58%) required an operation for resolution of small bowel obstruction. Two patients died from overwhelming sepsis. One of these two patients had radiation necrossis of the abdominal wall, and the other died of infection from a central venous catheter placed for hyperalimentation.

Obstruction associated with malignancy. Serosal or mesenteric involvement of the small bowel by malignancy was the major factor in 22 patients (32%) with small bowel obstruction. Intra-abdominal disease was confirmed by clinical impression, subsequent laparotomy, or autopsy. Ovarian cancer was responsible for 77% of these cases. Two patients had complete obstruction of the small bowel. Eighteen patients had had previous operations, and two patients with disseminated cervical carcinoma had previously been treated with pelvic radiation therapy.

The mean duration of tube drainage in 20 patients was 11.3 days (range, 2 to 32 days). Two patients went home with Cantor tube decompression and central hyperalimentation (for 59 and for 97 days).

Bowel function was restored for at least 30 days in seven patients (32%). Six of these patients died of disease within 10 months, and one patient is alive with disease. Of the other 15 patients, three died in the hospital (terminal care), two went home with the Cantor tube (see above), and 10 were treated surgically.

Postoperative adhesions. Fourteen patients (20%) developed small bowel obstruction within 6 weeks after a pelvic operation. The mean postoperative time was

Table II. Response to Cantor tube decompression

	No. of patients		
Etiology	Success	Failure	
Radiation	12	21	
Malignancy	7	15	
Adhesions	12	2	
	31 (45%)	38 (55%)	

13.4 days (4 to 43 days). Six of these patients had previous radiation therapy, including treatment with intraperitoneal phosphorus in one patient. Surgical procedures included hysterectomy, exenteration, and cytoreduction and reassessment for ovarian carcinoma. These patients required drainage for the least amount of time, 7.5 days (range, 5 to 13 days).

Tube decompression and restoration of bowel function for postoperative small bowel obstruction was successful in 12 patients (86%). One patient required laparotomy for lysis of adhesions. The second patient developed small bowel obstruction after pelvic exenteration. She required reinsertion of a second Cantor tube 12 days after removal of the first one, and subsequently had no further problems.

The etiologic factors for small bowel obstruction in this series of 69 patients are summarized in Table I. Radiation injury accounted for nearly 50% of all cases. The mean age of the patients was 55.6 years (range, 36 to 78 years), with no real difference among the three groups.

The Cantor tube passed through the duodenum the first time in 48 patients (70%). Two attempts were required in 20 patients (29%), and three attempts in one patient. In two patients, the tube failed to pass into the jejunum. Both had been admitted for terminal care management, and died within 8 days. Autopsies confirmed compression of the upper jejunum by metastases.

Parenteral nutrition, either central or peripheral, was used in 61 patients (88%).

In this series of 69 patients, 31 (45%) had successful decompression of the small bowel obstruction and restoration of bowel function with a Cantor tube (Table II). Patients with postoperative adhesions had the best response.

No major complications occurred relative to the use of the Cantor tube. One tube became obstructed, could not be irrigated, and was subsequently replaced by a nasogastric tube. One patient developed an earache which was attributed to the Cantor tube, and which resolved spontaneously within 10 days.

Comment

Dissatisfied with the double-lumen Miller-Abbot tube, Cantor,⁸ in 1946, described the single-lumen tube

used for intestinal decompression. He devised the single-lumen tube with a larger inner diameter and an increased number of holes for better decompression, and to prevent easy occlusion of the tubal lumen. It is for these reasons that we prefer the Cantor tube when small bowel decompression is indicated. The efficacy of the Cantor tube has also been reported in other large series of patients with small bowel obstruction.9

Partial or incomplete obstruction of the small bowel due to radiation injury, malignant disease, or postoperative adhesions is usually progressive, does not lead to strangulation, and is not considered to be a surgical emergency.5, 6, 10, 11 We think that, under these conditions, long tube decompression is indicated. In this series, 31 of 69 patients (45%) with small bowel obstruction were successfully treated with a Cantor tube. Eighty-six percent of patients with postoperative adhesions responded, and these results compare favorably with the 65% to 75% success rate of Peetz et al.9 and Smith et al.12 Likewise, Becker2 asserts that acute adhesive obstruction (within 4 weeks of an operation) can usually be safely and effectively treated by conservative therapy, and our data confirm this conclusion.

The management of our patients was not hindered by prolonged tube decompression, and no cases of strangulated small bowel obstruction were found in those patients who subsequently underwent an operation. Equally encouraging was the successful passage of the Cantor tube through the duodenum on the first attempt in 70% of the cases. In rare instances (and not required in this series of 69 patients), fluoroscopic guidance of the tube into the duodenum may be needed.

When radiation injury or intraperitoneal malignancy is the primary etiologic factor in small bowel obstruction, a much smaller percentage of patients will respond to conservative management.5,6,11 Our data also support this observation.

Discontinuation of Cantor tube decompression and surgical intervention should be considered in the following situations: (1) persistence of abdominal distention, cramping, nausea, or vomiting with the tube clamped; (2) radiographic confirmation of complete obstruction of the small bowel (failure of contrast medium to pass the site of obstruction); (3) presence of strangulating small bowel obstruction.

Major complications related to the use of the Cantor tube did not occur in this series of patients. Often, there is concern about the role of mercury in these tubes. Metallic mercury is harmless to the gastrointestinal tract, and the oral ingestion of mercury to treat small bowel obstruction has been reported since the 1600s.13 The fluidity, innocuous nature, and weight of mercury ideally suit it for use in intestinal tubes. However, rupture of the balloon and spillage of the mercury into the peritoneal cavity (via occult bowel perforation or anastomotic leak) could lead to persistent fistula or systemic toxicity.13, 14 Aspiration of mercury when the tube is removed has also been reported. Although one fatality was mentioned, most authors15 have not reported any deleterious effects.

Intussusception of the small bowel can occur. Contributing factors include fixation of the proximal tube to the nose, with "telescoping" of the bowel around the distal tube. 16 and too rapid removal of the tube, with subsequent jejunal intussusception.

The Cantor tube was shown to be safe, easy to use, and effective in the treatment of small bowel obstruction in this series of gynecologic patients. We think that long tube decompression and hyperalimentation should compose the initial management of partial or incomplete obstruction of the small bowel, since a significant percentage of these patients will not require a subsequent operation.

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Human myometrial adrenergic receptors during pregnancy: Identification of the α -adrenergic receptor by [${}^{3}H$] dihydroergocryptine binding

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The radioactive α -adrenergic antagonist [³H] dihydroerge-cryptine binds to particulate preparations of term pregnant human myometrium in a manner compatible with binding to the α -adrenergic receptor (α -receptor). [³H] Dihydroergocryptine binds with high affinity ($K_D=2$ nmol/ \bot and low capacity (receptor concentration = 100 fmol/mg of protein). Adrenergic agenists compete for [³H] dihydroergocryptine binding sites stereo-selectively ([-]-norepinephrine is 100 times as potent as [+]-norepinephrine) and in a manner compatible with α -adrenergic potencies (epinephrine \cong morepinephrine >> isoproterenol). Studies in which prazosin, an α_1 -antagonist, and yohimbine, and α_2 -antagonist, competed for [³H] dihydroergocryptine binding sites in human myometrium indicated that approximately 70% are α_2 -receptors and that 30% are α_1 -receptors. [³H] dihydroergocryptine binding to human myometrial membrane particulate provides an important tool with which to study the molecular mechanisms of uterine α -adrenergic response. (AM J OBSTET GYNECOL 1985;152:680-4.)

Key words: α-Adrenergic receptors, dihydroergocryptine, pregnancy, myometrium

The pregnant human uterus responds to α-adrenergic stimulation with contraction,1 thus indicating the presence of α-adrenergic receptors (α-receptors). However, the concentration, binding characteristics, and subtype distribution of these receptors have not been established. We previously characterized the \beta-adrenergic receptor in human myometrial particulate preparations with use of the β-adrenergic antagonist [3H] dihydroalprenolol.2 To characterize α-adrenergic banding to pregnant human myometrium, we examined the binding of [3H] dihydroergocryptine, a nonsubtype selective α-adrenergic antagonist,3 to myometrial membrane particulate. We report that [8H] dihydroergocryptine binds to myometrial particulate in a mariner consistent with binding to the \alpha-receptor, and that subtypes of α -receptors can be identified.

Material and methods

Material. [8H] Dihydroergocryptine with a specific activity of 22 to 31 Ci/mmol was obtained from New

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**Recipient of National Institutes of Health Research Career Development Award HD00267. England Nuclear. Phentolamine was a gift from Ciba Pharmaceuticals, and stereoisomers of norepinephrine, from Winthrop Laboratories. Other drugs and chemicals were from commercial sources.

Particulate preparation. Myometrium was obtained from the hysterotomy site at the time of cesarean section. In one case, myometrium was obtained from the hysterotomy site, the posterior lower uterine segment, and the uterine fundus in a woman who underwent cesarean hysterectomy in early labor for carcinoma in situ. Informed consent was obtained from all patients. Myometrium was dissected free of endometrium and serosa and quick frozen to -70° C. At the time of preparation of particulate, myometrium was thawed, minced, suspended in cold 50 mmol/L Tris HCl, pH 7.4, in a 2:1 (volume:weight) ratio, and homogenized with a Brinkman Polytron, at setting 8 for 15 seconds at 4° C, three times with cooling intervals of 1 minute. The homogenate was vacuum filtered at 4° C through two layers of cheesecloth. The filtrate was centrifuged at 29,000 × g for 15 minutes. The resultant pellet was resuspended in cold 50 mmol/L Tris HCl and centrifuged again at $29,000 \times g$ for 15 minutes. The final pellet was suspended in 50 mmol/L Tris HCl at a protein concentration of 3 to 5 mg/ml, rapidly frozen, and stored at -70° C until used for [3H] dihydroergocryptine binding studies. Binding of [3H] dihydroergocryptine was similar in particulates prepared from unfrozen or frozen myometrium and in those stored frozen for up to 4 months. Particulate protein concentration was

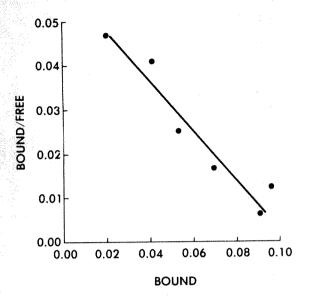


Fig. 1. Scatchard data array of specific [3H] dihydroergocryptine binding to human myometrium. Bound is specific bound [3H] dihydroergocryptine; free is free [3H] dihydroergocryptine. Data are from a representative experiment. Each point is the mean of duplicate determinations, and the line is computer-generated, determined by parameters resulting in the best fit of bound versus free [3H] dihydroergocryptine, which demonstrated interactions with a single class of sites. Kn equals 1.8 nmol/L and α-receptor concentration (R_T) is 104 fmol/mg protein. In seven experiments with different myometrial particulate, K_D was 2.30 ± 0.62 nmol/L, and R_T was 100 ± 16 fmol/mg protein (mean ± SD).

determined by the method of Bradford,4 with bovine serum albumin as the standard.

Binding assays. Experiments to determine dissociation constant (K_n) and receptor concentration were performed with seven concentrations of [8H] dihydroergocryptine, from 0.1 to 20 nmol/L. Competition experiments were performed with 3 nmol/L [3H] dihydroergocryptine (1.5 K_D) and increasing concentrations of competitors. Final assay volume was 0.25 ml and contained 1 mg/ml membrane particulate protein, [3H] dihydroergocryptine in 50 mmol/L Tris HCl, pH 7.4, 0.4 mmol/L ascorbic acid, 2% ethanol, 0.1 mmol/ L HCl, and competing adrenergic agents. Ethanol in a final concentration of 2% had no effect on [3H] dihydroergocryptine binding. Samples were incubated at 30° C for 30 minutes. The incubation was stopped by adding 5 ml of 50 mmol/L Tris HCl, pH 7.4, at 4° C and immediately filtering samples over Whatman GF/ C filters to separate bound from free radioligand. Filters were washed with an additional 15 ml of cold 50 mmol/L Tris HCl under low vacuum (1 ml/sec).

Data analysis. Specific binding was defined as binding prevented by 10 µmol/L of phentolamine. Receptor concentration and dissociation constant (KD) were de-

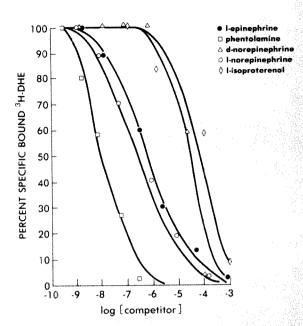


Fig. 2. Competition for specific [3H] dihydroergocryptine (3H-DHE) binding by adrenergic agents. Shown are results of experiments in which fixed concentrations of [3H] dihydroergocryptine and myometrial particulate were incubated with different concentrations of adrenergic agents. Data points are means of at least two experiments with different myometrial particulate. Inhibition constants (K1) for each adrenergic agent are shown in Table I.

termined by computerized analysis of specific bound versus free [3H] dihydroergocryptine. An iterative curve-fitting program5 was used to best fit data points weighted to minimize the effect of experimental error at different radioligand concentrations.6 The percentages of a - and a - receptors present in human myometrium were determined by analyzing the competition of α_{1} - and α_{2} -antagonists for [3H] dihydroergocryptine binding⁷ by means of an iterative, nonlinear, curvefitting program prepared for a Hewlett Packard 9825B computer.5 Statistical analysis of the goodness of fit for one and two affinity interactions was performed. A two affinity fit was accepted if comparison of the residual variances of the curves best fit by one and two affinities resulted in an F statistic such that p < 0.05.8 In experiments in which a two affinity fit was accepted, an estimate of the affinity of the high (Khigh) and low (Klow) affinity sites could be obtained. Because receptor concentration was kept low under these assay conditions, total ligand was very nearly equal to free ligand, and the inhibition constant (K1) could be determined for each competing adrenergic agent by the relationship described by Cheng and Prusoff9:

$$K_1 = I_{50}/[1 + (L_F/K_D)]$$

where I_{50} = the concentration of competing adrenergic agent which inhibits binding of [3H] dihydroergocryp-

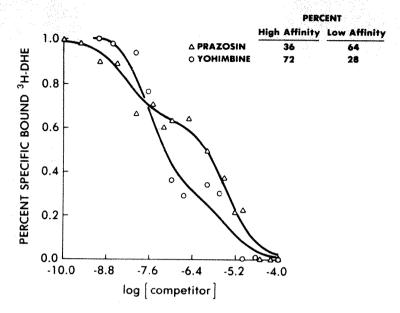


Fig. 3. Competition for specific [3H] dihydroergocryptine (3H -DHE) binding by subtype selective α -antagonists. In separate experiments, pooled uterine membranes from nine patients were incubated with [3H] dihydroergocryptine and prazosin (α_1 selective) or yohimbine (α_2 selective). Data points are means of duplicates, and curves are computer-generated. Yohimbine competed for [3H] dihydroergocryptine binding with two affinities (K_{high} 8.4 nmol/L; K_{low} 1209 nmol/L), as did prazosin (K_{high} 2.3 nmol/L; K_{low} 1386 nmol/L). Calculated percentages of receptors competed for with high affinity (α_1 for prazosin, α_2 for yohimbine) and low affinity (α_2 for prazosin, α_1 for yohimbine) are shown.

Table I. Inhibitory constants (K_t) of adrenergic agents for [³H] dihydroergocryptine binding to human and rabbit myometrial particulate

Competitor	Human (nmol/L)	Rabbit (nmoi/L)³
Phentolamine	4	15
<i>l</i> -Epinephrine	210	650
l-Norepinephrine	180	230
d-Norepinephrine	15,000	20,000
l-Isoproterenol	37,000	43,000

tine by 50%, L_F = the concentration of [3 H] dihydroergocryptine used in the assay, and K_D = the dissociation constant of [3 H] dihydroergocryptine for its binding sites. The percentages of the high and low affinity forms were calculated as previously described. The percentage of α -receptors competed with high and low affinity by a subtype specific agent indicates the percentage of subtype specific receptors of the same and different subtype, respectively, of the specific agent used.

Results

[³H] Dihydroergocryptine binding. [³H] Dihydroergocryptine binding in the presence of 10^{-5} mol/L phentolamine indicated that 50% of total [³H] dihydroergocryptine binding was specific binding to the α -receptor.

[³H] Dihydroergocryptine bound to human myometrial membrane particulates with high affinity ($K_D = 2.30 \pm 0.62 \text{ nmol/L}$) and saturably (Fig. 1). Binding was to a single class of receptors with a binding site concentration of $100 \pm 16 \text{ fmol/mg}$ of protein. [³H] Dihydroergocryptine binding sites were competed for stereoselectively (Fig. 2). The pharmacologically active (-)-stereoisomer of norepinephrine was 100 times as potent a competitor as the inactive (+)-stereoisomer. Adrenergic agonists competed for [³H] dihydroergocryptine binding in a manner compatible with α -adrenergic binding interactions and known pharmacologic potencies [Table 1: epinephrine ($K_1 = 210 \text{ nmol/L}$) \cong norepinephrine ($K_1 = 180 \text{ nmol/L}$) >> isoproterenol ($K_1 = 37,000 \text{ nmol/L}$)].

α-Receptor subtypes. To determine the proportion of α_1 - and α_2 -receptors in the myometrial particulate, we examined the competition of yohimbine, and α_2 -antagonist, and prazosin, an α_1 - antagonist, for [8 H] dihydroergocryptine binding sites (Fig. 3). Yohimbine competition isotherms were complex and best fit by a two affinity interaction (K_{high} 8.4 nmol/L; K_{low} 1209 nmol/L). Calculation of the percentage of bound [8 H] dihydroergocryptine competed for by yohimbine with high affinity (α_2 -receptors) was 72%; the percentage competed for with low affinity (α_1 -receptors) was 28%. Prazosin competed for [8 H] dihydroergocryptine binding with two affinities (K_{high} 2.3 nmol/L; K_{low} 1386 nmol/

L). The percentage of bound [3H] dihydroergocryptine competed for by prazosin with high affinity (a1-receptors) was 36%, and the percentage competed for with low affinity (α2-receptors) was 64%. These data demonstrate the expected reciprocal relationship between high and low affinity prazosin and yohimbine competition for specific [3H] dihydroergocryptine binding sites and show that at term approximately 70% of αadrenergic binding sites are of the α2-subtype and 30% are of the α_1 -subtype.

Comment

Human myometrium is responsive to α-adrenergic agents, and our data show that [3H] dihydroergocryptine binds to particulate preparations of pregnant human myometrium with interactions consistent with binding to the a-adrenergic receptor. Binding is high affinity, saturable, and stereoselective. Competition studies with adrenergic agonists showed the expected α-adrenergic rank order for inhibition of [3H] dihydroergocryptine binding. The KD, receptor concentration, subtype distribution, and half maximum inhibitory concentrations of different adrenergic agents for [8H] dihydroergocryptine binding to human myometrial particulates in these studies are similar to values determined in our laboratory10 and by others3 for nonpregnant rabbit myometrium. Particulate prepared from myometrium in several different uterine areas in one patient who underwent cesarean hysterectomy showed a variation of 27% (fundus, 175 fmol/mg protein; anterior lower segment, 127 fmol/mg protein; posterior lower segment 155 fmol/mg protein) in α-receptor concentration, thus indicating that α-receptor concentration in the lower uterine segment is similar to that in the more contractile upper uterine segment.

We found in these specimens that approximately 70% of the α-receptors present are α2-receptors and that 30% are α₁-receptors. The functional significance of the subtype distribution of α-receptors in human myometrium is not known. Stimulation of both subtypes is thought to increase uterine contractility,11,12 but a great deal of evidence suggests that this effect is mediated by different molecular mechanisms. a-1-Receptors, which are selectively bound with high affinity by the antagonist prazosin, are located postsynaptically in adrenergic target tissues, and produce their observed effects by modulation of intracellular calcium (Ca++) flux. Free intracellular Ca++ interacts with calmodulin to activate myosin light chain kinase, with resultant phosphorylation of the light chain of myosin. Myosin then interacts with actin and results in muscle contraction. Evidence exists that there are two mechanisms of α₁-mediated intracellular Ca⁺⁺ increase. The first is associated with tonic contraction and involves movement of Ca++ across cell membranes, presumably through

the opening of Ca++ channels. This mechanism is dependent on extracellular Ca++ and is reproduced by Ca++ ionophores. The second mechanism is associated with phasic contraction and involves the mobilization of Ca++ from intracellular stores. This α1 mechanism is not dependent on extracellular Ca++ or mimicked by Ca++ ionophores. α2-Receptors are selectively bound with high affinity by the antagonist yohimbine, and are located presynaptically in neural tissue and postsynaptically in target tissues, such as myometrium. Stimulation of postsynaptic myometrial \(\alpha_2\)-receptors is thought to produce uterine contraction by acting to reduce the concentration of intracellular cyclic adenosine monophosphate, probably through inhibitory effects on a regulatory component, N-protein. Since cyclic adenosine monophosphate acts to inhibit both the mobilization of intracellular Ca++ and the stimulation of myosin light chain kinase by Ca++, a reduction in the intracellular concentration of cyclic adenosine monophosphate will enhance uterine contractility. Our data indicate that stimulation of myometrial contractility through both a-receptor subtypes is possible in human uterus at term.

Myometrial α-adrenergic responses may be important in several currently obscure aspects of obstetric physiology and pathophysiology, including myometrial control of uterine blood flow, initiation of parturition, and changes in uterine responsiveness in preeclampsia. Whether changes in α-receptor concentration, specific α-receptor subtypes, ratio of α-receptor to β-receptor, or α-receptor agonist affinity or efficacy might also be important remains to be determined. The ability to examine directly a-receptors in human myometrium and quantitate α-receptor subtypes provides a tool which may be used in investigations of α-adrenergic sensitivity and its control, the localization of α -receptors within specific myometrial areas, and the role of areceptor subtypes in uterine function.

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Sonographic findings in severe preeclampsia twenty-four hours prior to clinical signs

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We describe a patient who had abnormal sonographic findings of the liver 24 hours before signs and symptoms of severe preeclampsia and HELLP syndrome (hemolysis, elevated liver enzymes, and low platelet count). The abnormal sonographic appearance of the liver prompted further investigation and was instrumental in the management of this case. (AM J OBSTET GYNECOL 1985;152:684-5.)

Key words: Sonogram, preeclampsia, abnormal liver parenchyma

Severe preeclampsia is classically characterized by a varying combination of the following signs and symptoms: high blood pressure, proteinuria, oliguria, cerebral or visual disturbances, and pulmonary edema. Recently, Weinstein described additional findings in severe preeclampsia to which he has given the epynom HELLP, the abnormalities of which include hemolysis, elevated liver function tests, and a low platelet count. By the time most of the clinical findings are apparent, the patient is already seriously ill. In this report, we describe a patient who had an abnormal sonogram of the liver 24 hours before any signs or symptoms of severe preeclampsia appeared. The abnormal sonographic findings in the liver prompted further investigation.

Case report

L. M., a 31-year-old woman (gravida 7, para 1, spontaneous abortions 5), had several early sonograms dur-

ing the present pregnancy because of her previous history of spontaneous abortions. Her past medical history was unremarkable except for gallstones. At 32 weeks, she developed pain in the right upper quadrant, as well as nausea; at that time she had a normal blood pressure, liver function tests, and amylase. The following week, a sonogram was obtained which included the gallbladder because of the persistent pain in the right upper quadrant.

The sonogram revealed a single active fetus in vertex position, with a normal amount of amniotic fluid and an estimate of fetal weight which was at the tenth percentile for 33 weeks. The sonogram of the gallbladder revealed gallstones and a markedly abnormal texture of the liver, consisting of multiple bright echogenic masses throughout the parenchyma (Fig. 1). After sonography, the patient was hospitalized because of the pain in the right upper quadrant which was thought to be secondary to cholecystitis. At that time, the blood pressure and platelet count were normal, although the values on the liver function tests were markedly elevated. Because of the abnormal texture of the liver, the patient was observed carefully. Twenty-four hours after the sonogram had been obtained, the blood pressure was elevated (160/120 mm Hg) for the first time, and the platelet count was depressed to 120,000. Later the same day, the platelet count fell to 27,000, and the patient developed proteinuria. A lecithin/sphingomye-

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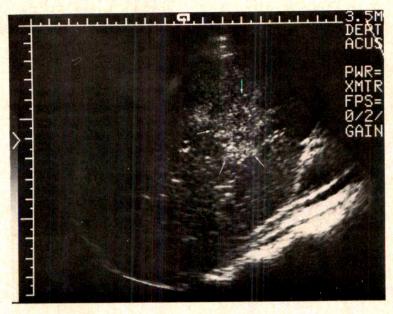


Fig. 1. Sonogram of the right lobe of the liver showing the heterogeneous texture of the parenchyma, and a bright echogenic mass (arrows).

lin ratio was consistent with immature lungs; however, because of maternal indications, labor was induced and delivery was effected that day.

Some pain persisted in the right upper quadrant, and the patient underwent a computerized tomographic scan of the upper abdomen 1 week after delivery. Gallstones were again noted, but the liver parenchyma was normal in texture. The patient subsequently underwent a cholecystectomy, with resolution of the pain in the right upper quadrant. At the time of operation, the liver was normal to inspection and palpation.

Comment

The syndrome of hemolysis, elevated liver enzymes, and low platelet count (HELLP syndrome) is associated with severe hypertension in pregnancy, as described by Weinstein.1 It has been well established in the past that patients with preeclampsia/eclampsia can develop periportal fibrin deposition with hemorrhage and necrosis during the course of their disease.2 The case reported here demonstrates that a change in liver parenchyma, probably representing periportal necrosis and hemorrhage, can precede clinical signs of the disease by at least 24 hours, and, in this case, was the index finding that led to the appropriate management for the patient.

Anecdotally, in our institution, recently, two other cases have occurred in which similar sonographic liver findings were present in women who already had the HELLP syndrome clinically. Because of the similarity between those earlier two cases and the present patient, the diagnosis of severe preeclampsia was entertained by the ultrasonographer at the time of the initial scan in this case; therefore, the liver sonogram was instrumental in the management of the patient. The fact that the liver of this patient was sonographically abnormal prior to the development of signs and symptoms suggests that, when such a sonographic finding is seen in a pregnant woman, the association with preeclampsia/ eclampsia should not be overlooked.

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Fetal heart rate accelerations and fetal movements in twin pregnancies

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The rate of fetal heart rate accelerations associated with fetal movements to total fetal movements of twin pregnancies was found to be significantly lower than that of pregnancies with singleton infants. The number of fetal heart rate accelerations was similar. As fetal heart rate accelerations are reflective of fetal movements, the results indicate increased fetal activity in twins that is related to an additive effect of two normally active fetuses. (Am J OBSTET GYNECOL 1985;152:686-7.)

Key words: Fetal movements, twins, fetal heart rate accelerations

The ratio of fetal heart rate accelerations associated with fetal movements to total fetal movements has been shown to increase with advancing gestation in singleton pregnancies.1 The association of fetal heart rate accelerations and fetal movements has not been studied in twins, although fetal activity in twins was documented previously and shown to be higher than that in singletons.

In the present study we have compared the ratio of fetal heart rate accelerations associated with fetal movements to total fetal movements and the number of fetal heart rate accelerations in singleton and twin pregnancies.

Thirty-four normal twin pregnancies were studied at 28 to 40 weeks of gestation. All had favorable perinatal outcomes. Dates were determined clinically and verified post partum according to Dubowitz's criteria.

In each patient a continuous fetal heart rate recording was performed. A mark button was used by the women to indicate on the continuous recording whenever fetal movements were felt.

The duration of monitoring was 20 minutes unless the fetal heart rate was not reactive, in which case monitoring was prolonged. A total of 411 tracings were evaluated. Accelerations were defined as fetal heart rate increases of at least 15 bpm for a duration of ≥15 seconds. The ratio percentage of fetal heart rate accelerations associated with fetal movements to total fetal movements for a 20-minute "window" of monitoring was calculated for each recording. The weekly average of this ratio was calculated for each woman and from this the final mean weekly ratio of fetal heart rate accelerations with fetal movements to total fetal move-

ments was obtained for all the study population of twins. This was compared with the mean weekly ratio of fetal heart rate accelerations with fetal movements to total fetal movements obtained in normal singleton pregnancies and reported separately.1 Similarly, in both

Table I. Statistical analysis data of the mean

weekly ratio of fetal heart rate accelerations

associated with fetal movements to total fetal

movements in twin and singleton pregnancies

Singleton

12

18

27

21

36

31

46

46

56

68

36

33

Mean (%)

21.4

22.5

41.3

37.3

42.7

46.8

54.5

64.2

59.0

63.5

64.0

68.3

69 5

NS

NS

NS (<0.1)

< 0.02

NS (<0.1)

< 0.01

< 0.01

< 0.01

< 0.01

< 0.01

< 0.05

NS

NS

Twins

6

10

17

18

18

19

21

10

Mean (%)

10.6

17.1

18.0

20.0

27.4

26.1

29.0

40.8

35.2

43.4

47.7

53.2

Week of

gestation

28

29

30

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singleton and twin pregnancies the mean weekly average of fetal heart rate accelerations in 20 minutes of monitoring was calculated and compared. For statistical

analysis the Student t test was used.

The results demonstrated a rising ratio of accelerations to total fetal movements with increasing gestational age. This ratio was significantly lower in twins compared with singletons (Fig. 1, Table I). The mean weekly average of fetal heart rate accelerations was similar in singleton and twin pregnancies. In both groups the number of accelerations per 20 minutes increased with advancing gestation (Fig. 2).

The finding of a lower ratio of fetal heart rate accelerations to fetal movements in twins compared with that in singletons, together with the similarity in the

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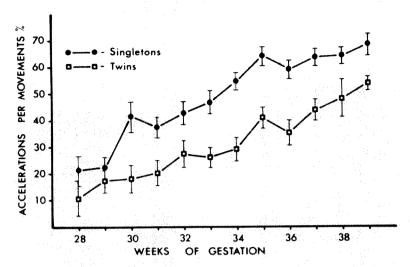


Fig. 1. The percentage ratio (weekly mean ± SE) of fetal heart rate accelerations associated with fetal movements to total fetal movements according to gestational age in twin and singleton pregnancies.

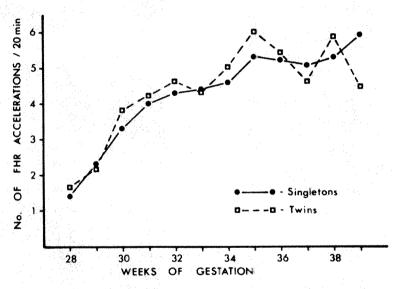


Fig. 2. The number of fetal heart rate (FHR) accelerations per 20 minutes according to gestational age in twin and singleton pregnancies.

number of accelerations in both groups, suggests that the lower ratio in twins is due to a greater number of perceived fetal movements. Furthermore since fetal heart rate accelerations signify fetal movements, even when unaccompanied by supportive maternal perception,² the similarity in accelerations suggests that fetal activity of one fetus of either singleton or twin pregnancies is the same. The higher rate of maternally perceived fetal movements in twin pregnancies can therefore be ascribed to an additive contribution of the two normally active fetuses.

The frequency of fetal heart rate accelerations as well as the ratio of fetal heart rate accelerations associated with fetal movements to total fetal movements was increased throughout the pregnancies of both our twin and singleton groups. Similar findings were documented previously only for singleton pregnancies and were ascribed to increasing maturation and integration of high levels in the central nervous system. These factors should be considered when the nonstress test of both singleton and twin pregnancies is interpreted.

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Isolated endometriosis in an inguinal hernia

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Presented is the case of a patient with inguinal endometriosis adjacent to a hernia sac in whom laparoscopy revealed no evidence of pelvic endometriosis. (AM J OBSTET GYNECOL 1985;152:688-9.)

Key words: Endometriosis, inguinal hernia, round ligaments, laparoscopy

Endometriosis has been reported in the inguinal canal in lymphatics¹ and once previously as nonlymphatic content of an inguinal hernia.² Whether such patients have coexisting pelvic endometriosis has not been reported. We report the case of a patient with inguinal endometriosis in whom laparoscopy revealed no evidence of pelvic endometriosis.

Case report

A 24-year-old woman, para 0-0-2-0, complained of noncyclic inguinal pain and swelling on the right side for 3 weeks. Menses were normal and a diaphragm was used for contraception. Examination revealed a tender, immobile, 3 by 3 cm mass in the medial aspect of the right groin. Findings on pelvic examination were within normal limits, except for a 2 by 2 cm cyst in the left anterior fornix. Sonographic examination of the pelvis

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produced negative findings. A complete blood count was within normal limits (white blood cell count, 5,000/ ml; hematocrit, 38%), and the erythrocyte sedimentation rate was 17 mm/hr. The presumptive diagnosis was right inguinal lymphadenopathy of uncertain origin, and the patient was taken to the operating room for exploration of the right groin. An indirect inguinal hernia was found, associated with a firm, hemorrhagic, reddish-brown, 2 by 2 cm mass. Frozen section revealed endometriosis. Final slides showed endometrial glands and stroma with hemorrhage and fibrosis. No lymphatic tissue was noted (Fig. 1). Twelve weeks later, the patient was returned to the operating room for laparoscopy and excision of the vaginal cyst. Laparoscopy revealed a normal uterus, with normal tubes and ovaries. No endometriosis or pelvic adhesions were present. All peritoneal reflections were normal, as were the round ligaments. The vaginal cyst, when excised, was found to be lined with low cuboidal epithelium, compatible with a Gartner's duct cyst.

Comment

Classic theories for the explanation of aberrant endometrium include retrograde menstruation, coelomic



Fig. 1. Endometrial tissue in fibromuscular stroma adjacent to hernia sac. (Hematoxylin and eosin. ×65.)

metaplasia, and lymphatic and vascular dissemination. Endometriosis in the inguinal region is difficult to explain if it is not in lymph nodes. Direct extension along the round ligaments from preexisting pelvic endometriosis is one possibility. The presence in this patient of endometrial tissue in a fibromuscular stroma adjacent to an inguinal hernia sac suggested that possibility. If coexisting pelvic endometriosis could be demonstrated, then a rationale would exist for suppressive therapy with Danocrine. Since Danocrine is not without side effects, one should look for signs of pelvic endometriosis endoscopically in patients with extrapelvic implants. If such signs are present, then treatment may be indicated. This patient demonstrated that endometriosis can exist in the inguinal region associated with a hernia sac without there being laparoscopic evidence of coexisting pelvic endometriosis. An isolated inguinal focus, such as that seen in this patient, could result, theoretically, from either retrograde menstruation, coelomic metaplasia, or lymphatic or vascular dissemination. Direct extension along the round ligaments from coexisting pelvic endometriosis, however, does not seem to be the case. Prophylactic use of Danocrine in such cases would not appear to be necessary unless laparoscopy demonstrated coexisting pelvic endometriosis.

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Diagnosis of congenital syphilis by immunofluorescence following fetal death in utero

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The etiology of fetal death may be difficult to identify, particularly with the presence of marked fetal maceration and autolysis. Immunofluorescence with human antitreponemal antibody was used in this case to establish a diagnosis of congenital syphilis. (AM J OBSTET GYNECOL 1985;152:689-90.)

Key words: Congenital syphilis, fetal death, immunofluorescence

Congenital syphilis is a rare but preventable cause of neonatal morbidity and mortality. The postmortem diagnosis of intrauterine fetal syphilitic infection depends upon the identification of spirochetes in affected fetal organs. When fetal death in utero occurs more than 24 hours before delivery, autolysis of the fetus and placenta may produce such nonspecific changes that the presence of a specific etiologic agent is masked. In the present case, which resulted in a macerated and autolyzed stillborn infant, a diagnosis of congenital syphilis was made by indirect immunofluorescence examination of frozen sections of the fetal liver stained with human antitreponemal antibody.

A 22-year-old white woman, gravida 4, para 3, presented at 40 weeks' gestation with a complaint of no

fetal movement for approximately 2 weeks. No fetal heart tones were auscultated and a subsequent sonogram revealed findings consistent with fetal death in utero. The VDRL and fluorescent treponemal antibody test results were positive. Labor was induced with oxytocin, and a stillborn 1700 gm female infant with breech presentation was delivered without difficulty. There was generalized maceration of the skin. No radiographic abnormalities were identified in a skeletal survey and there were no obvious stigmas of congenital syphilis. Except for enlargement of the liver, all other organ systems appeared well developed and organ weights were consistent with a gestational age of 36 weeks. There was marked autolysis of the viscera. No placental abnormalities except autolysis were identified. Microscopic examination of routine specimens from the fetus demonstrated generalized autolysis with total absence of nuclei. No histologic evidence of syphilis such as visceral fibrosis or perivasculitis were identified. Examination of the placenta demonstrated the presence of numerous polymorphonuclear leukocytes beneath the chorionic membrane and showed in a single section a focal aggregation of plasma cells. Numerous tissues were studied with the silver impregnation tech-

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Fig. 1. Spirochetes demonstrated by immunofluorescence (arrow).

nique of Nieto. Only the liver unequivocally demonstrated the presence of spirochetes.

Frozen sections of fetal liver were fixed to glass slides with acetone. The sections were incubated with human antitreponemal antiserum, which is provided as the positive control antiserum in the fluorescent treponemal antibody absorption assay system. Following a rinse with phosphate-buffered saline solution, the slides were incubated with rabbit antihuman immunoglobulin. The sections were then examined with the ultraviolet microscope. Fluorescent spirochetes were present in the fetal liver as demonstrated in Fig. 1.

The diagnosis of congenital syphilis in the stillborn infant requires the identification of spirochetes in fetal

tissue. This may be especially difficult when autolysis and maceration are present as was the case for this patient. Generally, histologic findings are compatible with the presence of a fetal syphilitic infection in such cases. These may range from histologic evidence of focal villitis and proliferative endarteritis with perivasculitis of fetal stem arteries in the placenta to classic findings of osteochondritis and periostitis in the fetus.1 In addition, the gross stigmas of congenital syphilis are readily identified. None of these findings were present for our patient. In the absence of these findings the diagnosis relies on the use of silver impregnation stains with routine histologic sections. These techniques are technically difficult and often yield inconsistent and artifactual results because of nonspecific background staining. Indirect immunofluorescence usually requires frozen tissue sections because antigenicity may be lost or altered by the standard fixation and embedding techniques. Direct immunofluorescence to identify spirochetes in sections fixed in Bouin fixative and embedded in paraffin has been reported1; however, this direct method is costly because it requires conjugation of the primary antibody with a fluorescent tagged molecule. Recently, Beckett and Bigbee² have localized Treponema pallidum by means of indirect immunoperoxidase-labeled antibodies. Their method would have been useful in this case, but the primary antibody prepared in rabbits is not generally available. By using indirect immunofluorescence we were able to make a diagnosis from completely autolyzed material that would otherwise have provided no useful diagnostic information. The use of standard technology in a novel way may provide the diagnosis of clinical disease in unusual circumstances.

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The morphologic characteristics of cervical ripening induced by the hormones relaxin and prostaglandin $F_{2\alpha}$ in a rabbit model

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In previous studies, both purified porcine relaxin and prostaglandin $F_{2\alpha}$ have been applied vaginally in the human to promote cervical ripening near term. In this study, the histologic changes in the cervix induced by these locally applied hormones are described in a rabbit model. Similar histologic changes occurred following treatment with relaxin or prostaglandin $F_{2\alpha}$ and these changes were comparable with those seen in the cervix following the spontaneous onset of labor in control rabbits. The main histologic features were a dissolution of the collagen bundles and an apparent increase in the ground substance. However, a unique giant cell infiltrate was seen in the relaxin-treated rabbits and the control rabbits in spontaneous labor. The nature and possible function of these giant cells are discussed. The similarity of the general morphologic changes in the cervix induced by relaxin and prostaglandin $F_{2\alpha}$ supports the concept that these hormones may act (either in sequence or separately) to activate the same collagenolytic system to produce the same effect in cervical connective tissue rather than act in parallel to produce separate or complementary structural changes. (AM J OBSTET GYNECOL 1985;152:691-6.)

Key words: Cervix, morphology, relaxin, prostaglandin F₂₀, rabbit

In most mammals, prior to parturition there are structural changes in the connective tissue of the cervix that lead to its altered distensibility, dilatation, and softening; these changes are collectively known as cervical ripening. In the human, several hormonal agents, including vaginally applied purified porcine relaxin² and prostaglandin $F_{2\alpha}$, have been given prior to the induction of labor to induce such changes in the cervix. These two hormones individually applied appear to produce a similar clinical effect, and when they are given in combination, there does not appear to be any additive effect. This suggests that these hormones may act in sequence or separately, to produce the same effect rather than work in parallel to produce different or complementary changes in the cervix.

No study of the histologic changes in the cervix following administration of pure porcine relaxin or prostaglandin $F_{2\alpha}$ has been published. This study examines and compares the histologic changes induced by these hormones. For ethical reasons adequate material for the study could not be collected from the human. Therefore the rabbit was chosen because it has been shown that this animal is a good model for the investigation of the mechanical behavior of the cervix and because the rabbit cervix appears to be comparable to the human cervix in its physiologic responses. ⁵

Material and methods

Twelve mature female New Zealand White rabbits, weighing 4.5 to 5.8 kg, were studied. Six of these served as untreated controls. Two of the control rabbits were not pregnant; two were put to death on day 28 of pregnancy, one was put to death in labor on day 29 of gestation, and one 12 hours post partum on day 30 of gestation. Of the other six rabbits, two were treated with 4 mg of prostaglandin F_{2a} and four with 0.3 mg of purified porcine relaxin. Both hormones were mixed in a tylose gel and administered vaginally by means of a syringe and catheter on day 27 of pregnancy. Fifteen hours later on day 28 of pregnancy the treated animals were sacrificed. The uterus was removed after sacrifice and the cervix and lower uterine segment fixed for

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Fig. 1. Cervical tissue from treated rabbits at day 28 of pregnancy. (Masson's trichrome stain.) A, Prostaglandin $F_{2\alpha}$ treated. (Original magnification \times 80.) B, Relaxin treated. (Original magnification \times 80.) C, Prostaglandin $F_{2\alpha}$ treated. (Original magnification \times 200.) D, Relaxin treated. (Original magnification \times 200.)

histologic examination. Paraffin sections of each cervix were cut in a sagittal plane and were stained with hematoxylin and eosin, Masson's trichrome (to highlight collagen), or alcian blue plus periodic acid—Schiff (PAS), which stains the mucopolysaccharides of the ground substance blue.

Tissues for electron microscopy were fixed in a 5% paraformaldehyde and 3% glutaraldehyde in cacodylate buffer, postfixed in 1.5% osmic acid, dehydrated in a series of ethanolic solutions, transferred to propylene oxide, and then embedded in Epon. Sections were examined on grids with a AE1801 electron microscope at initial magnifications of 1700 to 40,000.

The doses of prostaglandin $F_{2\alpha}$ and porcine relaxin chosen were the same in proportion to body weight as

the clinically effective dose of these hormones that produces cervical ripening when administered vaginally in the human.⁴ The relaxin used was obtained from the ovaries of pregnant sows and was purified by the method of Sherwood and O'Byrne,⁷ with modifications as described by Walsh and Niall.⁸ It had a biopotency of 1500 guinea pig units (GPU) per milligram relative to the reference preparation NIH-R-PI (potency 3000 GPU/mg).

Results

Control rabbits. The nonpregnant rabbit cervix was a relatively small, firm structure and the histologic features reflected this appearance in that the collagen, staining dark blue on Masson's stain, was densely

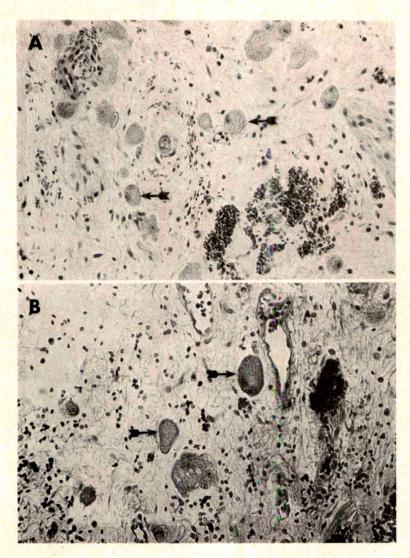


Fig. 2. Giant cells in rabbit cervical tissue (arrows). (Original magnification \times 200.) A, Control rabbits in spontaneous labor. (Hematoxylin and cosin stain.) B, Relaxin-treated rabbit. (Masson's trichrome stain.)

packed around the small muscle bundles, which stained pink. There was little ground substance between the collagen fibers, and the collagen bundles ran in relative unison around the cervical muscle.

In late pregnancy (day 28) the rabbit cervix was a larger, softer structure than the nonpregnant cervix, being about twice its previous size. The collagen bundles were not so densely packed and this resulted in a lighter blue staining of these fibers. The collagen bundles still ran together in a uniform fashion around the muscle fibers and there was an apparent slight increase in the ground substance.

In labor the rabbit cervix was even softer and longer than in late gestation. There was marked submucosal edema and separation of the collagen fibers deeper in the cervix. The collagen fibers seemed to be in disarray, with little uniformity in their direction. There was an apparent large increase in the ground substance, and the blood vessels were more prominent. Giant cells, which are described in detail below, appeared for the first time. They were seen more commonly around the blood vessels and in the subepithelial layer, although they could be found throughout the depth of the cervix and extended into the lower uterine segment.

Twelve hours post partum the rabbit cervix was still macroscopically large (approximately three times the nonpregnant size) but firmer in consistency than during labor. The muscle bundles were more prominent and a large number of blood vessels were apparent. The collagen fibers between the muscle bundles were more densely packed than during labor and were beginning to regain their uniformity of direction. There appeared to be a corresponding reduction of ground substance, and the giant cells seen in the cervix during labor could only occasionally be found.

Prostaglandin- and relaxin-treated rabbits (Fig. 1). With the exception of the more frequent presence of giant cells in the relaxin-treated cervices, the histologic

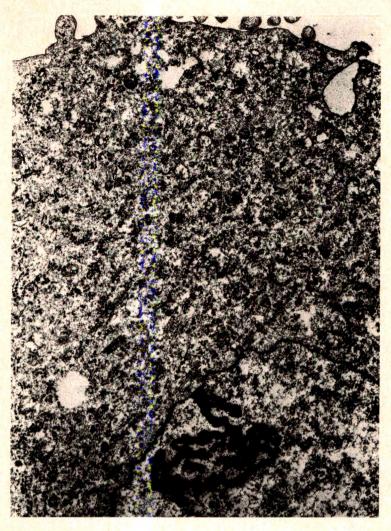


Fig. 3. Ultrastructure of a giant cell in the cervical tissue of a pregnant rabbit treated with relaxin on day 28 of gestation. (Original magnification × 7500.)

changes induced by the two hormonal treatments were similar to each other and comparable to the changes seen in the cervix of the control rabbit in labor. All hormonally treated cervices responded in similar fashion. Macroscopically the cervices were large and soft. Histologically there was submucosal edema, and deeper in the cervical tissue there was an apparent marked increase in the ground substance, as demonstrated with alcian blue-PAS stain. The collagen fibers did not run in uniform bundles but were generally widely separated without concurrence in their orientation. The blood vessels were more prominent in number and size, suggesting an increase in vascularity. Only an occasional giant cell was seen in one of the prostaglandin-treated cervices, whereas numerous giant cells were seen in three of the four relaxin-treated cervices. They were similar in morphologic features and general position to those seen in the cervices of laboring and postpartum control rabbits.

Giant cells (Figs. 2 and 3). Giant cells did not conform to a precise description of any previously recorded cells and could not be positively classified by any of four senior pathologists to whom they were shown. In this report we have simply called them giant cells because of their size (50 to 100 mµ in diameter) rather than definitively classifying them in any family of cells. Their appearance was somewhat similar to the appearance of ganglion cells and decidual cells, but neither neuronal nor uterine origin could be confirmed. They stained basophilic on hematoxylin and eosin staining and were PAS positive. They were multinucleated with large areas of vacuolation in the cytoplasm (Fig. 2, A and B). Ultrastructural examination showed these cells to be densely packed with ribosomes, mitochondria, and rough-surfaced endoplasmic reticulum (Fig. 3). Numerous small vesicles were seen throughdout the cytoplasm but Golgi apparatus was not prominent and there were very few lysosomes. Multiple microvilli covered the surface of the cell, and vesicles near the cell membrane suggested active pinocytosis. In some cells the nucleolus was relatively amorphous and in others the nucleolonema stood out clearly from the pars amorpha. Overall the ultrastructural picture was that of a very active cell possibly involved in chemical or hormonal production and secretion.

These cells were seen frequently in the relaxintreated cervices and in the control cervix during labor. Only very occasional giant cells were seen in one of the prostaglandin-treated cervices and in the postpartum control cervix. They were not seen in any of the non-pregnant or 28-day-pregnant control rabbits. When present, giant cells could be found throughout the connective tissue of the cervix but they appeared to be more prominent around blood vessels and in the submucosal layer. These cells were also found to extend into the lower uterine segments of the relaxin-treated and laboring rabbits. In the latter sections, giant cells were found predominantly in the deeper layers of the decidua and between the muscle fibers of the inner myometrium.

Comment

This study shows that vaginally applied porcine relaxin and prostaglandin $F_{2\alpha}$, applied separately, induce similar histologic changes in the rabbit cervix. In c.inical trials where porcine relaxin and prostaglandin F_{2e} were used in combination to induce cervical ripening, no additive clinical effect or apparent synergism in their action was found.4 This histologic study supports the suggestion made in these clinical trials that relaxin and prostaglandin F2a may act to produce the same structural changes in the term cervix rather than acting in parallel to produce separate or complementary changes in cervical connective tissue. Although it is possible that relaxin and prostaglandins may act in sequence to produce this similar effect, it is also possible that these hormones may separately stimulate the same collagenolytic process in cervical tissue near term. It has recently been shown that both relaxin9 and prostaglandin10 increase cyclic adenosine 3',5'-monophosphate in human cervical tissue at the end of pregnancy and it has been suggested that the effects of these hormones on cervical connective tissue metabolism are mediated by cyclic adenosine monophosphate. Thus, the results of this histologic study and previous clinical studies suggest that relaxin and prostaglandin F_{2a} may have similar morphologic effects on the term cervix, even though the link or sequence of action between them is yet to be established.

The treatment-induced histologic changes in the rabbit cervix were similar to those observed in the rabbit cervix during spontaneous labor, suggesting that relaxin and prostaglandin $F_{2\alpha}$ can induce the normal structural changes in the cervix associated with spontaneous cervical ripening and parturition. The hormonally induced changes described in this paper are comparable to the morphologic changes in needle biopsies of the human cervix in early pregnancy, following treatment with prostaglandin E2.11 However, there have been no reports of histologic changes in the human cervix at term after treatment with prostaglandin $F_{2\alpha}$ or relaxin, presumably because of the difficulty in obtaining adequate cervical tissue at this time. Since the rabbit has been shown to be a suitable model for studying cervical ripening,5 it is suggested that the connective tissue changes induced by these hormones in the rabbit cervix, as described in this paper, are likely to reflect the morphologic changes that occur in the human cervix after vaginal application of prostaglandin F2a or relaxin.

The nature and function of the giant cell infiltrate is of some interest. The giant cells were mostly confined to the cervical and lower uterine segment sections from the rabbit in labor and three of the four rabbits treated with relaxin. Although these particular cells do not seem to have been described before in such a situation, different types of cells have been seen to invade the cervix during parturition in several species. Leukocytic infiltration has been described in the ovine cervix during parturition.12 These latter cells are mostly neutrophils, but plasma cells, eosinophils, and lymphocytes are also seen. In the guinea pig and the human there is a leukocytic invasion of the cervix prior to parturition, with a significant increase in the number of eosinophils.13 Junqueira et al.14 hypothesize that the neutrophilic polymorphonuclear leukocytic invasion of the human cervix around the time of parturition contributes to the widespread collagenolysis occurring in the cervix at that time. They suggested that this action is mediated through the release of collagenase either from the leukocytes or indirectly from other cervical cells producing enzymes capable of digesting the extracellular matrix proteins. Other studies15 show that macrophages are another potential source of such enzymes and that macrophage plasminogen activator promotes digestion of the extracellular matrix. Whether the giant cells described in the rabbit cervix near parturition are involved in collagenolysis cannot be determined from this study.

The ultrastructural photographs were taken by members of the Department of Pathology, The Queen Elizabeth Hospital, Adelaide. We extend thanks to Dr. Helen Chambers, Senior Pathologist, The Queen Victoria Hospital, Adelaide, for her expert histological advice and to Mr. Kim Tank for the printing of the illustrations.

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Experimental evidence for the progress of labor with the increase in the force of cervical dilatation after rupture of the membranes

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To examine the mechanical forces involved in cervical distention, actual tensions of the fetal membranes up to the bursting level were measured experimentally with the use of five circular membrane holders that simulate various cervical dilatations (radii 1 to 5 cm). The results show that the force of cervical dilatation with the head alone increases remarkably as the cervix dilates, and this force is always larger than that when the membranes are intact. Thus the membranes appear to interfere with effective cervical dilatation and progress of labor after fixation of the fetal head to the cervix. (AM J OBSTET GYNECOL 1985;152:696-704.)

Key words: Membrane tension, force of cervical cilatation, rupture of membranes

Delivery involves various factors that cannot be explained in terms of muscle contractility alone. Although, so far, much attention has been given to studies of uterine contractility, there are various other factors

to be studied; these include membrane tension, the forces of friction between the head (with or without membranes) and cervix, hydrostatic pressures, biochemical factors including changes in tissue viscoelasticity, and prostaglandins and other hormones. The study of one factor is by no means adequate, and integrated studies of several fields will promote and enable the understanding of the complicated physiology of delivery. It is also questionable to what extent theoretical experiments can be extrapolated to the living situations. With these limitations and assumptions in

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Reprint requests: Dr. Yukio Manabe, Department of Obstetrics and Gynecology, Kyoto University School of Medicine, Sakyo-Ku, Eyoto 606, Japan. mind, our present study examined one of the important factors in the physiology of delivery, that is, memorane tension as related to the vector force of cervical dilatation and uterine contractions.

We previously reported that the force of cervical dilatation theoretically increases after rupture of the membranes at an appropriate time, either artificially or spontaneously. In the present study, clinical values were obtained experimentally and applied to our mathematical formulas in order to validate the previous estimations. Inevitably, this study was performed under several assumptions; these include: The uterine contractile pressure is constant (60 mm Hg); cervical elasticity is constant; the head is a sphere with a 5 cm radius; the head and membranes are always in direct contact in the process of cervical dilatation; and the cervix and head with or without membranes make circular contact, although a broader area has been documented by Lindgren.⁸ More important, the actual forces of friction between the head (with or without membranes) and cervix and the hydrostatic pressure involved must be considered, but these factors were not included in our calculations because of mathematical and experimental limitations.

Since accurate values of membrane tension (T_m) under various conditions are important to calculate the actual force of cervical dilatation, we measured T_m experimentally with the use of ring-shaped models that simulate different cervical dilatation (r) and under increasing pressure (P).

The relationship in a membranous sphere may be expressed by Laplace's formula: 2T = PR, where T is the tension at any point on the surface; R, the radius of the sphere; and P, the pressure. In the calculation of T_m it is important to evaluate whether the human fetal membranes actually conform to this formula. Although many authors used this formula in their study of bursting tension of the membranes, they used a ringshaped apparatus with an arbitrarily selected radius (r) (0.45 cm,⁴ 3.75 cm,⁵ 3.0 cm,⁶ 1.35 cm,⁷ 2.5 cm^ε). The membrane tension as related to r (radius of cervical dilatation) and P (uterine contracting pressure) has not been investigated, despite the importance of such studies.4, 9

Material and methods

Fetal membranes from 76 normal women (39 to 41 gestational weeks) were studied. No consideration was given with regard to age or parity of the patients.4 Immediately after placental delivery the membranes were immersed in physiologic saline solution until testing (within 2 hours) to avoid dehydration. Labor was induced by amniotomy at a time when the cervical state was evaluated as being appropriate for induction. Membranes from women in whom rupture had oc-

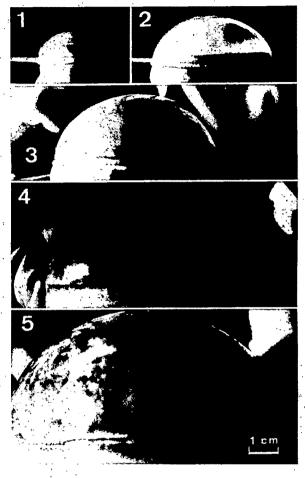


Fig. 1. Representative photographs which show the relationship between radius of the membrane holder (r) and deflection height of the membranes (h) under a constant pressure (P), 60 mm Hg. From top: 1, r = 0.9 cm, h = 0.5 cm; 2, r = 2.1cm, h = 1.5 cm; 3, r = 2.8 cm, h = 2.2 cm; 4, r = 4.1 cm, h = 3.3 cm; 5, r = 5.1 cm, h = 4.2 cm.

curred after the establishment of significant labor were excluded from the study, as they were assumed to be damaged because of physiologic stretching due to labor.7 Particular care was taken not to separate the chorion from the amnion since both tissues have their own tensile forces.6 Only the central part of the membranes, too close to neither the placenta nor the tear, was studied.[€]

The apparatus used for measuring membrane tension consisted of three parts, a membrane holder (a shallow, cylindrical, open-ended glass chamber), a mercury manometer, and an air source. These were connected to a T tube. Air introduction was controlled accurately by a valve, and transmitted to the membrane holder and manometer. Air was used instead of physiologic saline solution; it was found to have no effect on the physical properties of the membranes.⁶ The membranes with the chorion side down were positioned over the rim of the membrane holder and kept tightly

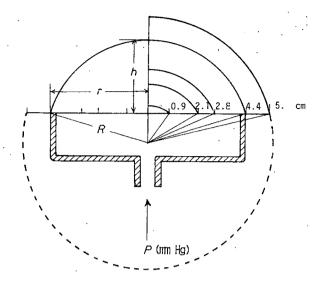


Fig. 2. Left half: Schematic representation of the relation between radius of the membrane holder (r) (in centimeers), deflection height of the membranes (h) (in centimeters) and radius of the distended sphere (R) (in centimeters). Right half: This shows that R is not constant but increases proporticulally with the increase of r, under a given pressure (P), 60 mm Hg in this figure. This figure was constructed based on the extual measurements of r and h and calculations of R (see Fig. 1).

in place with a broad cotton band. The region of comtact of the glass holder with the membranes was glazed so as to prevent slippage of the membranes. Air was introduced slowly until the membranes finally burst.

Devices similar to the one mentioned above have been used by several investigators to measure bursting tension of the membranes. However, unlike those authors we measured the membrane tension (T_m) in relation to both the changes in radius (r) of the membrane holder (equivalent to that of cervical opening) and the pressure (P) needed to distend the membranes (presumed to be uterine contractions).

Five membrane holders with r values of approximately 1, 2, 3, 4, and 5 cm were used. T_m was calculated with increasing P from 0 to more than 300 mm Hg under different r values. The inflation heights (h) ur der different P and r values were measured from 35 mm photographs taken at a fixed distance (30 cm) from the center of the membrane holder. A macrolens (Zuiko, focal distance 50 mm, Olympus Camera, Tokyo, Japan) was used. Bursting P was read on a pressure recorder. More than 600 photographs were enlarged to nearly the original size of the membrane holder to measure the inflated membranes at different P and r values (Fig. 1).

 T_m was calculated with the formula:

$$2T_m = PR \tag{1}$$

where R is radius of deflection (bubble) and P is pressure. As shown in Fig. 2, R is not constant; this differs from the case of cervical dilatation by the head or the

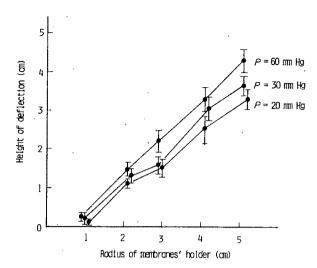


Fig. 3. Relation between radius of the membrane holder (r) and deflection height (h) under three different pressures (P): 20, 30, and 60 mm Hg. The values at 60 mm Hg correlate with Fig. 1, I to 5, and the right half of Fig. 2. Values are the mean \pm SD; n = 8 to 15.

head plus membranes where the radius of the curvature of the presenting part is constant (approximately 5 cm).

The left half of Fig. 2 shows that:

$$\sqrt{R^2 - r^2} + h = R \tag{2}$$

Therefore

$$R = \frac{r^2 + h^2}{2h}$$

where $h \le r$. Combining equations 1 and 2

$$T_m = \frac{PR}{2} = P \frac{r^2 + h^2}{4h} = \tag{3}$$

$$P \frac{r^2 + h^2}{4h} \times 981 \times 1.37 \text{ dynes/cm}$$

where P is in millimeters of mercury and r and h are in centimeters. With this equation the T_m at different r and P values was calculated.

Results

Membrane tension (T_m) calculated from various pressures (P) and radii (r) of the membrane holder. Fig. 3 demonstrates a linear relationship between r and h at three different P values, 20, 30, and 60 mm Hg. Coefficients of linear regression lines were r = 0.998, p < 0.001 (P = 20 mm Hg); r = 0.988, p < 0.005 (P = 30 mm Hg); r = 0.999, p < 0.001 (P = 60 mm Hg). This indicates that the T_m versus r relation in equation 3 is also linear.

The relation between the gradually increased P and r and the resulting changes of T_m is shown in Fig. 4. The bursting tensions of the membranes are also shown in Fig. 4. When r was smallest (0.9 cm) the T_m did not

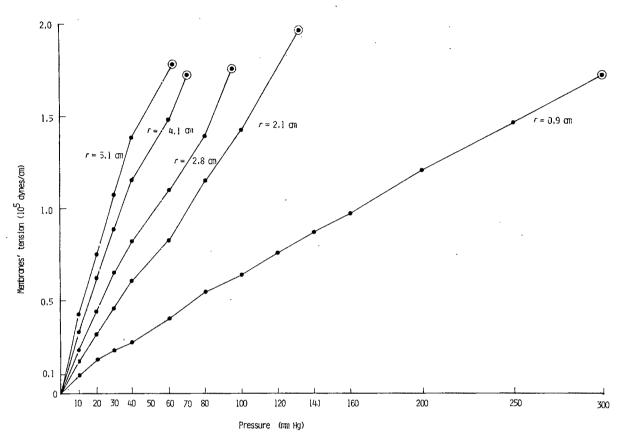


Fig. 4. Membrane tension (T_m) up to the bursting levels correlated with the changes in pressure (P)and radius of the holder (r). This figure indicates that bursting tension is not attained even with unphysiologically high pressure when the cervix is not dilated while it is attained with physiologic pressure when the cervix is greatly dilated. Bursting tensions (©) are nearly equal (1.70 × 10⁵ to 1.95×10^5 dynes/cm). Values are the means of eight to 15 experiments.

increase sharply although P rose steadily to >200 mm Hg. Thus, when P was in the order of 30 to 100 mm Hg, which is the normal range recorded in physiologic delivery,10 the bursting tension is never obtained. The bursting was observed in some cases only when extraordinary high P of >250 mm Hg was applied, and the bursting P was 298 \pm 43 mm Hg (mean \pm SD, n = 15). At this F the calculated T_m , on the average, was 1.70×10^5 dynes/cm.

With the increase of r of the holder to 2.1 and 2.8 cm, the bursting P values, 132 ± 26 mm Hg (n = 15) and 95 ± 23 mm Hg (n = 12), respectively, were attained more easily. At these pressures the respective average T_m values were 1.95×10^5 and 1.75×10^5 dynes/cm. When the r was increased further to 4.1 and 5.1 cm, the rise of T_m with increasing P was remarkable, and the average bursting tensions, 1.72×10^5 and 1.78 × 10⁵ dynes/cm, respectively, were obtained with bursting P values of 70 ± 15 mm Hg (n = 12) and 62 ± 10 mm Hg (n = 8), respectively. These bursting pressures are in the physiologic range of uterine pressure during normal delivery.

Fig. 4 therefore demonstrates that the bursting ten-

sions of the membranes are nearly the same, ranging from 1.70×10^5 to 1.95×10^5 dynes/cm with different P and r values, thus indicating the rationale of application of Laplace's law (2T = PR) to the human membranes. Figs. 5 and 6 were reconstructed based on the data shown in Fig. 4. Fig. 5 shows the relation between T_m and r at various P values. The bursting P versus rrelationship is given in Fig. 6; this curve also confirms that the formula 2T = PR is applicable to human membranes.

Force of cervical distention by the fetal head (W_b) . With cervical dilatation by the fetal membranes alone, the radius of deflected bubble curvature changes (see Figs. 1 and 2); however, the radius (R) of curvature of the fetal head (presenting part) is constant (Fig. 7).

As described in our previous study! the force of cervical dilatation by the fetal head (W_h) is:

$$W_h = \frac{F}{\pi} \frac{1}{\tan \theta} = \frac{F}{\pi} \frac{r}{\sqrt{R^2 - r^2}} \text{ (dynes)}$$
 (4)

However

$$F = \pi r^2 P$$
 (dynes, where P is in dynes/cm²) (5)

Combining equations 4 and 5, we get

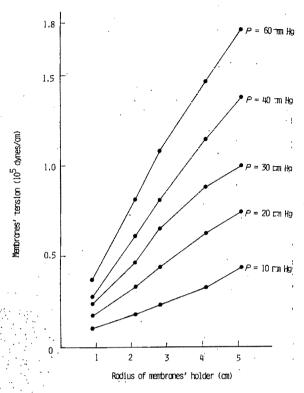


Fig. 5. Membrane tension (T_m) correlated with the changes of radius of the holder (r) and pressure (P). High T_m is attained with relatively low P as r increases. Values are the mean of 8 to 15 experiments.

$$W_h = \frac{P \cdot r^3}{\sqrt{R^2 - r^2}} \text{ (dynes)} \tag{6}$$

Under the increasing r, P was assumed constant, 60 mm Hg/cm = $60 \times 981 \times 1.37 = 0.81 \times 10^5$ dynes/ cm2, as the average pressure during the first and second stages of delivery. Also, the R of the fetal head i assumed constant (5 cm); with this constant R the cervix is gradually forced to dilate. Therefore, W_h values in Table I and the W_h curve (see Fig. 9) were obtaine \exists by substituting respective values in equation 6. This curve indicates that W_h (stretching) increases sharply with the progress of cervical dilatation. It is obvious from the form of W_h also (see Fig. 9) that W_h is an increasing monotonic function of r, which tends to infinity as rtends to R. The physical reason for the rapid increase in the force of distention as $r \cong R$ is that the curva ure of the head causes it to act like a wedge of angle approaching zero with a mechanical advantage approach-

Force of cervical distention by the fetal membranes plus head (W_m) . As shown schematically in Fig. 8, the cervix is dilated with constant R, since it is assumed that the head is in direct contact with the membranes. The formula to express W_m (membranes plus head) has been calculated previously and is:

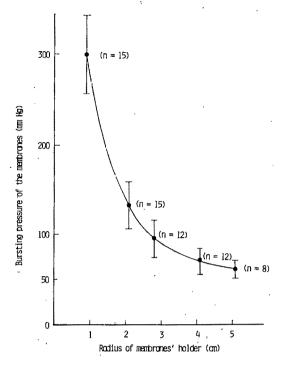


Fig. 6. Bursting pressures correlated with radius of the holder (r). This curve indicates that the membranes rupture with physiologic uterine pressure (40 to 100 mm Hg) when the cervix dilates. Values are the mean \pm SD. Number of experiments in parentheses.

$$W_{m} = \frac{2rT_{m}}{\sqrt{R^{2} - r^{2} \cdot R}} \cdot \left(\sqrt{\frac{FR}{2\pi T_{m}}} - r\right)$$

$$\left(\sqrt{\frac{FR}{2\pi T_{m}}} + r\right) \text{ (dynes)}$$
(7)

Combining equations 5, 6, and 7 we get

$$W_m = \frac{PR - 2T_m}{R} \frac{r^3}{\sqrt{R^2 - r^2}} \text{ (dynes)} =$$
 (8)

$$\frac{P \cdot r^3}{\sqrt{R^2 - r^2}} \cdot \left(1 - \frac{2T_m}{PR}\right) = W_h \cdot \left(1 - \frac{2T_m}{PR}\right) \text{ (dynes)}$$

As in the case of W_b , P was assumed constant: 60 mm Hg = 0.81×10^5 dynes/cm², and R = 5 cm; therefore, $PR = 4.03 \times 10^5$ dynes/cm. When 20 mm Hg (1/s of the total P) is considered as the membrane component of 60 mm Hg, the membrane tension (T_m) is:

$$T_{m1} = \frac{PR}{2} \times \frac{1}{3} = 0.67 \times 10^5 \text{ (dynes/cm) (constant)}$$

Similarly, when 30 mm Hg ($\frac{1}{2}$ of the total P) is assumed as the membrane component of 60 mm Hg, the membrane tension (T_{m2}) is

$$T_{m2} = \frac{PR}{2} \times \frac{1}{2} = 1.01 \times 10^5 \text{ (dynes/cm) (constant)}$$

Table I. Forces of cervical dilatation by the fetal head (W_h) and the membranes plus the head $(W_{m1}^* \text{ and } W_{m2}^\dagger)$ correlated with the radius of cervical opening (r)

			, u,			
г (<i>cm</i>)	W _h (10 ⁵ dynes)	PR (10 ⁵ dynes/cm)	$I - \frac{2T_{ml}}{PR}$	W _{mi} (10 ⁵ dynes)	$I - \frac{2T_{m2}}{PR}$	W _{m²} (10⁵ dynes)
1.0	0.16	4.03	0.67	0.11	0.50	0.08
1.5	0.57	4.03	0.67	0.38	0.50	0.29
2.0	1.41	4.03	0.67	0.94	0.50	0.70
2.5	2.91	4.03	0.67	1.94	0.50	1.45
3.0	5.44	4.03	0.67	3.63	0.50	2.72
3.5	9.68	4.03	0.67	6.46	0.50	4.84
4.0	17.20	4.03	0.67	11.47	0.50	8.60
4.5	33.72	4.03	0.67	22.48	0.50	16.86

Uterine pressure (P) was assumed constant: 60 mm Hg/cm². Radiu: of the head (R) was considered 5 cm.

*Membrane component of P was assumed to be ≥ 0 mm Hg, with resulting membrane tension (T_{ml}) being 0.67×10^5

†Membrane component of P was assumed to be $\stackrel{?}{=}0$ mm Hg, with resulting membrane tension (T_{m2}) being 1.01×10^{5} dynes/cm.

These values are nearly identical to T_m values (0.70 \times 105 and 0.98 × 105 dynes/cm) obtained in the experimental model (Fig. 5) at a P of 20 mm Hg (with 4.6 cm radius of membrane holder), and at 30 mm Hg (with 4.8 cm radius of holder), respectively, since with these radii of the membrane holder the radius of curvature becomes approximately 5 cm according to Fig. 3 and equation 2. This information indicates that the membranes themselves possess such a large inherent resistance in vivo.

 W_{m1} and W_{m2} (forces of cervical dilatation by the membranes plus head with T_{m1} and T_{m2} , respectively) in Table I and W_{m1} and W_{m2} curves in Fig. 9 were calculated by substituting the respective values in equation 3. If $(1 - 2T_n/PR)$ is constant, as assumed in Table I, W_n has characteristics similar to those of W_{h} .

It is clear from data in Table I and curves in Fig. 9 that when the membranes are in direct contact with the head, the force of cervical distention is always less than that without the membranes. It is also obvious from equation 8 that \dot{W}_m is less than W_h . For example, $W_h - W_{m2}$ is 0.75×10^5 dynes when cervical dilatation is 4 cm (r = 2 cm), and $\dot{W}_h - W_{m2}$ increases to 16.86×10^5 dynes as the cervical dilatation advances to 9 cm (r = 4.5 cm). Thus the membranes, having inherently strong tension, work as a hindrance to effective cervical dilatation, and this interference becomes more pronounced as the cervix dilates.

Evaluation of theoretical W_h and W_m curves. When the relation between W_h and r is considered as a function between $f(x)_1$ and x,

$$W_h = \frac{P \cdot r^3}{\sqrt{R^2 - r^2}}$$

is expressed as

$$f(x)_1 = \frac{P \cdot x^3}{\sqrt{R^2 - x^2}}$$

Then

$$f'(x)_1 = \frac{R \cdot x^2}{(R^2 - x^2)_2^{\frac{3}{2}}} (\sqrt{3}R - x)(\sqrt{3}R + x)$$

This is always positive since $0 \le x \le R$. When x approaches zero, f(x) also shifts to zero. Also, when x increases toward R, f(x) becomes large toward $+ \infty$ (posit ve infinity).

$$f'(x)_1 = \left[3PR^2x^2 (R^2 - x^2)^{\frac{-3}{2}} - Px^4(R^2 - x^2)^{\frac{-3}{2}} \right]' =$$

$$\frac{Px}{(R^2 - x^2)_{\frac{5}{2}}^{5}} \left[\left(x^2 - \frac{R^2}{2} \right)^2 + \frac{23}{4} R^4 \right] \ge \frac{23}{4} R^4 > 0$$

Therefore, this curve does not have the point of inflection.

Similarly, when the relation between W_m and r is considered as a function between f(x) and x,

$$W_m = \frac{\dot{P} \cdot r^3}{\sqrt{R^2 - r^2}} \left(1 - \frac{2T_n}{PR} \right)$$

is expressed as

$$f(x)_2 = \frac{P \cdot x^3}{\sqrt{R^2 - x^2}} \left(1 - \frac{2T_m}{PR} \right)$$

When the head and membranes are in direct contact. the R of curvature is constant. Since P is assumed to te 60 mm Hg, PR and $T_m = \frac{P_1 R}{9}$ are also constant, where P_1 is the component of P which works to the membranes and P_2 is that which works to the cervix, and $P_1 + P_2 = P$.

Therefore, $1 - \frac{2T_m}{PR}$ can be replaced by k (constant). Then,

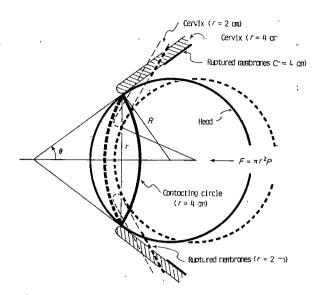


Fig. 7. Schematic representation of the force of cervical distention by the fetal head (W_h) alone. $F = \pi r^2 P$ and increases the direction of force to delivery by labor. R: Radius of subsere, that is, the fetal head, an assumed constant (5 cm). r: Ladius of contracting circle made between the head and cervix, which increases approximately 1 to 5 cm in this study. The convix is indicated by dotted and solid bars; each bar shows cervical dilatation of r = 2 and 4 cm, respectively. P: Uterine pressure, an assumed constant, that is, 60 mm Hg = 0.81×10^5 when 10^5 cm 10^5

$$f(x)_2 = \frac{k \cdot P \cdot x^3}{\sqrt{R^2 - x^2}} = k \cdot f(x)_1$$
$$f'(x)_2 = k \cdot f'(x)_1$$
$$f''(x)_2 = k \cdot f''(x)_1$$

This shows that the characteristics of both functions, $f(x)_1$ and $f(x)_2$, are similar, that is, a simple increase without limit and point of inflection.

Comment

Our mathematical model, with the several assumptions mentioned above, indicates that rupture of the membranes at an appropriate time theoretically results in the increase of the force of cervical distention by the elimination of membrane tension (T_m) . Fig. 9 condrms that the force of cervical dilatation by the head alone is always larger than that by the head covered with the membranes.

As shown in Figs. 4 to 6 and has been suggested by Keetel, the membranes (amnion and chorion) rupture when the cervix is almost fully dilated (r=4 or $5\,\text{mm}$), even when uterine contractions (P) are within the physiologic range (60 to 100 mm Hg). Conversely, the nembranes do not rupture when the cervix is relatively closed ($r=0.9\,\text{cm}$), even at high pressures (>200 mm Hg) not encountered during normal delivery. The latter fact is supported clinically; even when an extension

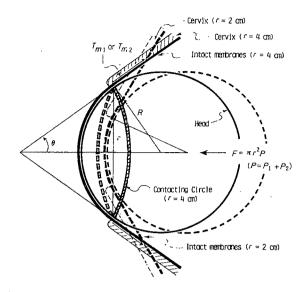


Fig. 8. Schematic representation of the force of cervical distention by the membranes plus head (W_m) . See the legend to Fig. 7 for F, R, r, P, and θ . R is constant because membranes are in direct contact with the head. P consists of two components: P_1 and P_2 , where P_1 works on the membranes and P_2 works on the cervix. In this study P_1 was assumed to be 20 or 30 mm Hg while total P was 60 mm Hg, that is, $P = P_1 + P_2 = 60$ mm Hg. T_{m1} and T_{m2} are membrane tensions based on these different P_1 values (20 or 30 mm Hg).

dinarily high uterine pressure is artificially produced by oxytocin or prostaglandins, rupture of the membranes cannot easily be attained when the cervix is nearly closed. In short, the more dilated the cervix, the less pressure required to burst the membranes.

In the previous study we also showed that if rupture of the membranes occurs too early, when the head is still floating over the pelvis, the difference of the forces of cervical distention before and after rupture of the membranes may become negative. The difference of the forces, however, becomes positive when the head dips and makes direct contact with the cervix through the membranes. In other words, before the head comes into direct contact with the cervix, the membranes (water bag) play an important role as a cervical dilator with inherent tension, that is, tension equivalent to that produced by the head after rupture of the membranes. This wedgelike role of the water bag in cervical dilatation is clearly seen in artificial abortion at the midtrimester¹¹ or in premature delivery. Since the presenting part of the fetus is small and the water bag is relatively large, an early rupture of the membranes results in a sudden reduction of the once-established uterine activity. Thus, despite the important function of the water bag in the beginning of labor, the membranes eventually interfere with the effective head-tocervix force because of the inherent tensions (T_m) after firm head engagement to the cervix.

In our present study, two important factors were not

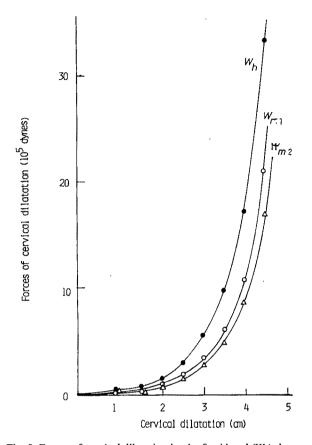


Fig. 9. Forces of cervical dilatation by the fetal head (W_b) alone and by the membranes plus the head (W_{m1} , where the membrane component of P was assumed to be 20 mm Hg, with resulting membrane tension ($T_{\rm ml}$) being 0.67×10^5 dynes/cm, and W_{m2} , where the membrane component of P was assumed to be 30 mm Hg, with resulting membrane tension (T_{m2}) being 1.01×10^5 dynes/cm.) correlated with cervical dilatation. It is evident that the membranes, with the tension, work as a deterrent of cervical dilatation. Uterine pressure (P) = 61 mm Hg; radius of head = 5 cm. Closed circles: W_h . Open circles. W_{ml} , with membrane component $P_1 = 20$ mm Hg. Open tricygles: W_{m2} , with membrane component $P_1 = 30$ mm Hg.

included in the calculations because of mathematical and experimental limitations, the hydrostatic pressures involved and the friction between the head (with or without membranes) and cervix. When the membranes precede the fetal head and are filled with fluic and when the head makes contact with the cervical rim, no fluid escapes past the fetal head up into the remainder of the uterine cavity or past the fetal head into the bulging forebag. Clinically, this may not necessarily be the case but must be taken into consideration as a parameter of the study that limits complete clinical interpretation. Second, a major physical force involved in the clinical situation is that of friction. The coefficient of friction probably changes when the membranes rupture. This change is caused by differences in the physical structure presenting to the cervical surface. Before the membranes rupture and the head is clearly descended the head is in contact with the membranes, not the cervix. The coefficient of friction between the cervix and the membranes may not be the same as that between the cervix and the head. Adaptive changes in the feta head produced by molding and soft tissue forces would enhance the forces between the head and cervix. All these changes would affect the normal vector against which the frictional forces are exerted.

Our current studies and information on the physiologic features of delivery are almost limited to the forces produced by the uterus in labor and in expelling the fetu:. As our attention was focused only on the role of memorane tension, the results obtained have limitations and are based on many assumptions; therefore, this approach alone cannot fully explain the various complicated and interrelated factors and forces involved in delivery. Among the important matters for further nvestigation, the forces of friction, as has been discussed before, as well as the hydrostatic pressure involved must be studied. A method for estimation of frictional forces generated in the pelvis perhaps would be pred ctive of the future progress of labor. Ir. addition, the interrelationships among such factors as forces of cervical distention and friction, cervical elasticity, uterine contractility, and the influence of hormones must also be studied. Since prostaglandins are released from the cervix under the influence of stretching by foreign bodies¹²⁻¹⁵ or amniotomy, ¹⁶ it is also probable that there are abundantly released by the strong friction between the head (with or without membranes) and the cervix during delivery. These studies are intriguing and indispensable for deepening our understanding of the physiology of delivery.

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Examination of the role of calcium in ovulation in the in vitro perfused rabbit ovary with use of ethyleneglycolbis(β-aminoethyl ether)-n,n'-tetraacetic acid and verapamil

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The in vitro perfused rabbit ovary preparation was used to examine the role of calcium in the ovulatory process. Two groups of rabbits were studied. In the first group, verapamil hydrochloride (10⁻⁴ mol/L), a calcium channel blocker, was used together with human chorionic gonadotropin (50 IU) in the perfusate. Verapamil had no apparent effect on human chorionic gonadotropin-induced ovulation. Verapamil treatment, however, significantly reduced the percentage of ovulated ova that were mature (68.8%) in comparison to ovulated ova from human chorionic gonadotropin-treated control ovaries (95.0%). In a second experimental group, ethyleneglycol-bis(β-aminoethyl ether)-n,n'-tetraacetic acid (2.0 mmol/L), a calcium ion chelator, was included in the perfusate with gonadotropin. The ethyleneglycol-bis(β-aminoethyl ether)-n,n'-tetraacetic acid significantly reduced ovulatory efficiency (16.7% ± 9.43%) in comparison to that of controls exposed to human chorionic gonadotropin alone (79.5% ± 11.1%). In addition, ovulation occurred at an earlier time in ovaries perfused with ethyleneglycol-bis(8-aminoethyl ether)-n.n'-tetraacetic acid; however, only four ovulations occurred in these ovar es. These four ovulated ova were immature, probably reflecting the early time of ovulation. Furthermore, both verapamil and ethyleneglycol-bis(βaminoethyl ether)-n,n'-tetraacetic acid blocked ovarian smooth muscle contractions during ovarian perfusion. These data provide additional support for the concept that calcium dynamics influence the processes of ovulation and ovum maturation. Furthermore ovarian smooth muscle contractions do not appear to be essential for ovulation in this model. (AM J CBSTET GYNECOL 1985;152:705-8.)

Key words: Ovulation, ovum maturation, calcium

The role of the calcium ion in the mammalian ovulatory process has been examined by several investigators. Ionic calcium is required for smooth muscle contractility, and ovarian smooth muscle contractions may be involved in the process of ovulation. Agents that block follicular wall smooth muscle contractions have been shown to inhibit ovulation in vivo in the hamster. Furthermore, in the rabbit, the calcium chelating agent ethylenediaminetetraacetic acid partially suppresses human chorionic gonadotropin (hCG)—induced ovulation during in vitro ovarian perfusion. In

contrast to these studies, ovarian perfusion in medium devoid of calcium and magnesium with or without gonadotropin results in ovulation despite the absence of detectable smooth muscle contractions.4

Calcium ion may also be involved in oocyte maturation. Verapamil, a calcium ion channel blocking agent which inhibits transmembrane calcium transport, not only blocks ovulation in vivo in the hamster but also suppresses oocyte maturation in vitro in the mouse.^{2, 5}

The present study was designed to examine further the role of calcium in ovulation by use of (1) verapamil, a calcium channel blocker, which prevents calcium from entering the cell, thereby decreasing the calcium concentration in the intracellular environment, and (2) ethyleneglycol-bis(β-aminoethyl ether)-n,n'-tetraacetic acid (EGTA), a calcium chelating agent, which binds extracellular calcium, thus altering the extracellular environment. With use of an in vitro perfused rabbit ovary preparation, the effects of these agents on follicle growth and rupture, oocyte maturation, and ovarian smooth muscle contractility were evaluated.

Material and methods

Sexually mature New Zealand White female rabbits were used in all experiments. Animals were isolated for

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Table I. The effect of verapamil hydrochloride on ovulation and ovum maturation in the in vitro perfused rabbit ovar-

	Verapamil (10 ⁻⁴ mol/L) plus hCG (50 IU)	Cc-trol, hCG '50 IU)
Ovulating ovaries (n)	5/6	Ξ/ε
Ovulatory efficiency (%)		
(mean ±SE)	69.3 ± 17.3	83.6 ± 9.16
Ovulation time (hr)		
$(mean \pm SE)$	7.81 = 0.61	7.62 ± 0.67
Ovulated ova	n = 16	n = 20
Germinal vesicle break-	- 68.8*	<u>⊆</u> =.(*
down (%)		
Degeneration (%)	50.0	E. (
Follicular oocytes	n = 6	n = 6
Germinal vesicle break-	- 83.3)C
down (%)		
Degeneration (%)	100	<u>)</u> نات
Ovarian water content (%	86.8 ± 0.91	87.3 ± 0.94

^{*}Significant difference at p < 0.05.

a minimum of 3 weeks prior to use under comrolled light and temperature with free access to Purina Rabbit Chow and water. Both ovaries of each rabbit were removed at the time of laparotomy, which was performed with the animals under pentobarbital sodium and the sia (32 mg/kg). Anastomotic connections to each ovary were ligated and the ovarian artery was canrulated in situ. Each ovary with its ovarian artery and ven and supporting adipose tissue was removed and placed in a separate perfusion chamber. In this model, the perfusion fluid consisted of tissue culture medium 199 (M. A. Bioproducts, Bethesda, Maryland) Explemented with insulin, heparin, penicillin, and szeptomycin. The perfusate (150 ml) was circulated at a rate of 1.5 ml/min and was continually oxygenated with a mixture of 95% oxygen and 5% carbon dioxide. Details of the cannulation technique and perfusion pro-edure have been previously described.6.7

Two experimental groups were included in this study. The first group, consisting of six rabbis, examined the effect of verapamil on hCG-induces ovulation and oocyte maturation. Verapamil hydrochloride, 10⁻⁴ mol/L (Knoll Pharmaceuticals, Whispany, New York), was incorporated in the perfusate of one ovary from each rabbit at the onset of perfusion. This concentration of verapamil has been shown to be effective in inhibiting ovulation in vivo in the hamster. The contralateral ovary was not exposed to verapamil and served as a control. An ovulation-inducing cose of hCG (50 IU) was added to the perfusate of both craries 30 minutes after the onset of perfusion.

In the second group, the calcium ion chelating agent EGTA (2.0 mmol/L) was included in the perfurate of one ovary from each of six rabbits. EGTA was not included in the perfusate of the contralateral contral ova-

ries. Thirty minutes after onset of perfusion, 50 IU of hCG was administered to the perfusion fluid of both ovaries.

In both groups ovarian perfusion was continued until 12 hours after initial exposure to the gonadotropin stimulus. Ovaries were periodically observed for evidence of follicle growth and/or rupture. The mean ovulatory efficiency was calculated for each treatment group (six ovaries). Ovulatory efficiency is defined as the percentage of mature follicles (>1.5 mm in diameter) that proceed to rupture during ovarian perfusion. When follicular rupture occurred, the ovum in its cumulus mass was carefully recovered from the ovarian surface by Pasteur pipette for microscopic evaluation. At the end of perfusion, mature unruptured follicles (>1.5 mm) were punctured and the follicular oocytes retrieved. Cumulus cells from both ovulated ova and follicular oocytes were removed with a solution of 0.5 mg/ml of hyaluronidase and gentle agitation with a narrow bore pipette. Both ovulated ova and follicular oocytes were placed on slides and fixed in 2.5% glutaraldehyde followed by 10% lacmoid in 45% acetic acid. All ova were examined microscopically. Ovum maturity was expressed as the percentage of ova with germinal vesicle breakdown. Germinal vesicle breakdown classification included those ova lacking an intact germinal vesicle that had progressed to the metaphase I or II stage of development. Ova were also assessed for signs of degeneration including vacuolation, necrosis, cytolysis, and loss of spherical shape. Following perfusion, ovaries were weighed (wet weight), dried at 80° C in a vacuum oven for 24 hours, and reweighed (dry weight). The percentage of ovarian water content was calculated as the percentage difference between wet and oven-dried ovarian weights and served as an index of ovarian edema. Data were analyzed statistically with the χ^2 test and Yates' correction or Student's t test.

Ovarian contraction studies. Six additional rabbits were used for the purpose of measuring ovarian smooth muscle contractions in vitro to determine if verapamil or EGTA would inhibit ovarian smooth muscle contractility. In three rabbits, the experimental protocol was identical to that of the first group, with one ovary perfused in medium containing verapamil and hCG and the contralateral ovary perfused with hCG alone. The ovaries of three additional rabbits were perfused as described in the second experimental group. One ovary was perfused with EGTA and hCG and the contralateral ovary was perfused with hCG alone. In all ovaries studied for smooth muscle contractions a 6-0 silk ligature was tied around the pole of the ovary nearest the fimbria and the ovary was connected to a Statham strain gauge/universal transducer leading to a Beckman type RM dynograph recorder. At the onset of perfusion, the tension supporting the ovary was adjusted to between 300 and 500 mg. The opposite pole of the ovary was connected by silk suture to a glass hook on the base of the perfusion chamber in order to stabilize the ovary. Ovarian smooth muscle contractions were recorded throughout the 12 hours of perfusion, and the occurrence of ovulation was noted. Details of the model for measuring ovarian smooth muscle contractions have been previously described.^{1,4}

Results

The results of the first group of experiments using verapamil are shown in Table I. Verapamil did not inhibit the number of ovulating ovaries or alter the ovulatory efficiency as compared to results with hCG treatment alone. The mean time of follicular rupture was similar in verapamil-treated and control ovaries. Verapamil treatment significantly reduced the percentage of ovulated ova achieving germinal vesicle breakdown when the results were compared to those in the controls (p < 0.05). The percentage of follicular oocytes with germinal vesicle breakdown, however, was not affected by verapamil treatment. In addition, the percentage of degenerated ova (ovulated and follicular) was similar in the verapamil-treated and the control ovaries. No difference in degree of ovarian water content was noted between the two groups.

In the second group, in comparison to the control ovaries, EGTA treatment reduced the number of ovulating ovaries and significantly inhibited ovulatory efficiency (p < 0.01) (Table II). Time of ovulation was significantly accelerated in EGTA-treated ovaries (p < 0.01). Ovulation occurred at a mean time of 3.44 ± 1.51 hours in EGTA-treated ovaries and 8.20 ± 0.55 hour in control ovaries. Only four ovulations occurred in the six EGTA-treated ovaries. The four ovulated ova were immature. No difference was found in the degree of maturity or degeneration of follicular oocytes or in the percentage of ovarian water content between the EGTA-treated ovaries and the controls.

Ovarian smooth muscle contractions could not be detected in any of three ovaries perfused with verapamil and hCG. In contrast, ovarian contractions were recorded in all three contralateral control ovaries perfused with hCG alone. In those ovaries perfused with EGTA and hCG, none of the three ovaries showed evidence of contractions, while in hCG-treated contralateral control ovaries smooth muscle contractions were recorded in two of three ovaries. In those ovaries in which smooth muscle contractility was observed, no correlation could be established between changes in amplitude and/or frequency of contractions and the time of ovulation occurrence.

Table II. The effect of EGTA on ovulation and ovum maturation in the in vitro perfused rabbit ovary

	EGTA (2.0 mmol/L) plus hCG (50 IU)	Control, hCG (50 IU)
Ovulating ovaries (n)	3/6	6/6
Ovulatory efficiency (%) (mean = SE)	16.7 ± 9.43*	79.5 ± 11.1*
Ovulation time (hr) (mean \equiv SE)	3.44 ± 1.51†	$8.20 \pm 0.55\dagger$
Ovulated ova	n = 4	n = 26
Germinal vesicle break- down (%)	0‡	96.2‡
Degeneration (%)	0	46.2
Follicular oocytes	n = 23	n = 5
Germinal vesicle break- down (%)	78.3	80.0
Degeneration (%)	82.6	0.08
Ovarian water content (%)	86.3 ± 0.58	85.1 ± 0.94

^{*}Significant difference at p < 0.01.

Comment

Both verapamil and EGTA inhibit ovarian smooth muscle contractions during in vitro perfusion. Verapamil does not inhibit hCG-induced ovulation but does appear to inhibit ovum maturation. In contrast, EGTA has an ovulation inhibitory effect. While EGTA also inhibits ovum maturation, this effect appears to be related to the accelerated time of ovulation in EGTA-treated ovaries and consequently provides less opportunity for ovarian exposure to gonadotropin.

Verapamil acts to decrease intracellular calcium concentration by inhibiting the passage of calcium through the cell membrane.8 This alteration in intracellular calcium may be responsible for the inhibition of ovum maturation observed in our study. Ovum maturation may be dependent on critical levels of intracellular calcium. In support of this concept, verapamil has been shown to inhibit polar body formation in cultured mouse oocytes.6 One might speculate, however, that verapamil acts to inhibit ovum maturation through an intrinsic property of the agent distinct from its effect on calcium flux. Our results are in contrast to the in vivo studies reported by Martin and Talbot,2 in which ovarian intrabursal injections of 25 to 50 mmol/L of verapamil inhibit in vivo ovulation in the hamster. Verapamil did not affect ovulation in these in vitro studies in the rabbit. Differences between the results of these studies may stem from variations in experimental design and species.

EGTA binds extracellular calcium, thus manipulating the calcium concentration in the extracellular environment. EGTA inhibits ovulation in vitro in the rabbit. This observation correlates with earlier studies with the

[†]Significant difference at p < 0.01.

[‡]Significant difference at p < 0.005.

in vitro perfused rabbit ovary model in which ethylenediaminetetraacetic acid was shown to have an inhibitory effect on hCG-induced ovulation. Extracellular calcium levels may, therefore, play a role in the process of follicular rupture. While EGTA has a much higher affinity for calcium than for other ions, it remains possible that the drug affects ovulation through its nonspecific chelating properties. In those instances in which ovulation occurred in EGTA-treated ovaries, it occurred earlier than is normally observed following hCG. Ovulation has also been demonstrated to occur consistently and at an advanced time during ovarian perfusion in Ca++/Mg++-free medium.

Ovarian smooth muscle contractions were suppressed by either verapamil or EGTA treament in vitro. hCG-Induced ovulation was not inhibiæd by verapamil but was blocked by EGTA. It is unlike that ovulation inhibition associated with EGTA is relead to blockade of ovarian smooth muscle contractility. Ovulation consistently occurs during ovarian perfusion in Ca++/Mg++-free medium with or without hCG: however, ovarian smooth muscle contractions cannot ze detected.4 In contradistinction to these studies, in cl ronic experiments in vivo and in acute experiments in vivo and in vitro, ovarian contractility was enhanced at the time of ovulation in the rabbit.9-11 Based on thes∈ data, although smooth muscle contractions may accompany ovulation, they may not represent an essential component of the ovulatory process.

Although results of these experiments are difficult to interpret, variations in calcium ion concentrations can alter the process of ovulation and ovum maturation in vitro. These effects appear to be independent any

requirements for calcium to promote ovarian smooth muscle contractility and may stem from a role for calcium in other cellular functions.

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Uterine and whole body oxygen extractions in the pregnant rabbit under chronic steady-state conditions

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Seventeen pregnant rabbits were studied under conscious unstressed conditions after catheterization of the right ventricle (RV), a femoral artery (A), and a uterine vein (UV). Respiratory gas tensions, pH, and oxygen saturations and contents were determined serially hroughout the latter half of gestation. The uterine coefficient of oxygen extraction increased with gestational age (R = 0.86) and became 60.6% ± 0.3% during the last 4 days of pregnancy. To compare uterine perfusion with whole body perfusion in relationship to oxygen demands, the (A₀₂ – JJ₀₂/A₀₂ – RV₀₂) ratio was computed. The ratio was ≥1 after 20 days of gestation, which demonstrated that in regard to oxygen demands the rabbit uterus is relatively underperfused compared to the rest of the maternal organism in the last part of pregnancy. A comparison with analogous data in other species demonstrates that the pregnant rabbit, like the guinea pig, has a much lower rate of uterine blood flow than does the pregnant sheep. These interspecies differences in the perfusion rate and oxygen extraction of the pregnant uterus are related to differences in placental structure. (AM J OBSTET GYNECCL 1985;152:709-15.)

Key words: Pregnant rabbit, uterine oxygen extraction, respiratory gases, uterine respiration, whole body oxygen extraction

Comparative observations in pregnant animals uncler normal physiologic conditions provide an approach to understanding how interspecies differences (e.g. differences in body weight, fetal metabolic demands, length of gestation, placental structure, etc.) influence the physiologic aspects of gestation. Specifically, we have been interested in how small mammals with a large litter and a labyrinthine placenta accommodate the cardiovascular demands of pregnancy.

The rabbit has a hemochorial labyrinthine placenta with the characteristics of a countercurrent exchanger, 1, 2 a large litter, a short gestation (31 day:), a relatively small fetomaternal weight ratio, and Dff-spring which are born immature and grow rapidly before weaning. Ninety-eight percent of fetal growth occurs in the last half of pregnancy. It is during this place of fetal development that we have addressed the question of whether the rabbit uterus is relatively underperfused or overperfused in relationship to other ma-

ternal tissues, and in comparison to the pregnant uterus of other species.

Recently, we developed techniques for chronic sampling of maternal arterial, uterine venous, and right ventricular blood in conscious pregnant rabbits. In this report, we present data on respiratory gases, pH, oxygen saturations, and oxygen contents which define normal physiologic values for this species both across the uterus and across the whole body, thus permitting a comparison with two other species (sheep and guinea pig) for which analogous information is available.

Material and methods

Seventeen New Zealand rabbits were artificially inseminated to achieve accurate dating (within 1 day). Anesthesia was induced with 150 mg of ketamine intramuscularly and 45 mg of Rompun (xylazine) intramuscularly. Portex epidural catheters (18 gauge) were placed in the right and left uterine veins by an indirect approach that involved retrograde catheterization of the femoral veins and threading of the catheter to the proper uterine vein by direct abdominal visualization.4 Polyurethane catheters (Micro Renethane 0.040 inch outer diameter by 0.025 inch internal diameter) were used in the right ventricle to decrease the incidence of blood clots. Placement of the right ventricular catheter was confirmed by monitoring pressure tracings as the catheter was advanced from the right jugular vein. A polyvinyl catheter (0.034 inch outer diameter by 0.023

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Table IA. Respiratory gases and pH data for arresial and right ventricular blood in pregnant rabbits

	N	þН	Pco ₂ (torr)	Oxygen capacity (mM)	PO ₂ (torr)	Oxygen saturation (%)
Maternal artery	56	7.427 ± 0.001	9.3 ± 0.3	6.4 ± 0.01	64.5 ± 0.1	94.4 ± 0.04
Right ventricle	48	7.425 ± 0.001	3.2 ± 0.05	6.5 ± 0.01	37.8 ± 0.1	64.6 ± 0.2

N = Total number of daily measurements for all animals.

Table IB. Respiratory gases and pH data for ut rine venous blood

Gestational age (wk)	No. of animals	pН	PCO ₂ (torr)	Po_2 (torr)	Oxygen saturation
. 14	1.	7.422	31.1	39.7	69.1
15	1	7.450	33.0	43.5	76.2
16	Ι.	7.422	35.2	46.8	71.7
17	4	7.442 ± 0.00 ;	32.7 ± 0.5	44.8 ± 2.5	70.9 ± 1.5
18	4	7.415 ± 0.01	32.8 ± 0.2	38.7 ± 0.6	66.5 ± 2.0
19	4	7.424 ± 0.001	34.7 ± 0.05	36.0 ± 0.7	63.2 ± 1.6
20	4	7.413 ± 0.00	35.0 ± 0.7	39.2 ± 0.4	62.5 ± 1.9
21	. 8	7.425 ± 0.00	35.1 ± 0.3	38.5 ± 0.8	60.1 ± 1.0
. 22	7	7.416 ± 0.00	35.5 ± 0.3	37.1 ± 0.5	56.2 ± 0.9
23	7	7.390 ± 0.00	37.3 ± 0.4	34.5 ± 0.4	52.6 ± 1.1
24	5	$7.379 \pm 0.00^{\circ}$	38.6 ± 0.8	31.8 ± 0.7	42.5 ± 2.5
25	7	7.360 ± 0.00	39.4 ± 0.3	31.0 ± 0.6	44.9 ± 1.4
26	4	7.383 ± 0.01 -	40.2 ± 1.4	29.8 ± 2.2	40.9 ± 5.6
27	10	7.375 ± 0.00 -	39.2 ± 0.5	29.6 ± 0.3	39.4 ± 0.7
- 28	10	$7.339 \pm 0.00^{\circ}$	42.6 ± 0.4	28.7 ± 0.3	37.2 ± 1.10
29	5	7.356 ± 0.002	42.9 ± 0.6	31.3 ± 0.7	40.7 ± 2.4
30	1	7.364	31.8	31.8	38.2
r =		-0.6316	0.6632	-0.6864	-0.8123
p =		< 0.001	< 0.001	< 0.001	< 0.001

Each number represents the mean for the given gentational age. SEM's were calculated by using the number of animals for each day.

inch internal diameter) was placed in the distal norta through the right femoral artery. All catheters were secured with suture and surgical adhesive (Eastman 910), tunneled subcutaneously to the area between the scapulae, and protected by a plastic cap sutured to the skin. The catheters were maintained patent by fluthing daily with a heparin saline solution (200 units/ml...

Postoperatively, the animals were kept in cages constructed to minimize reaction to daily laboratory reutine and were given food and water ad libitum. Studies were begun 3 days postoperatively when food intake had returned to normal levels (average daily food intake, 146 gm).

Each day, three sets of samples of blood were dawn simultaneously from each of the four catheters and analyzed for pH, PCO₂, and PO₂ with a Radiomete gas analyzer (model BMS 3 MK 2) and for oxygen capacity and oxygen saturation with a Radiometer Hemosinieter OSM2 at 38.5° C. The samples were analyzed within 10 minutes of sampling to avoid red blood cell sedimentation and other errors related to storage. The results from three sets of samples were averaged and used for that day of gestation. After 2 to 5 days (mean, 3.31 days of study), the animals were killed and catheter placement was documented.

Calculations. Coefficients of oxygen extraction -zere

calculated across the pregnant uterus and the whole body as:

$$\frac{A_{O2} - V_{O2}}{A_{O2}} \times 100$$

where A_{02} and V_{02} represent arterial and venous oxygen content, respectively. To compare whole body perfusion to uterine perfusion in regard to oxygen demands, the ratios of their coefficients of extraction were used, which simplifies to

$$\frac{(A_{O2}-V_{O2})}{(A_{O2}-V_{O2})}$$
 whole body

Statistics. Regression analysis was carried out by means of the standard least-squares method.¹⁷ The calculated r values and their significance are included in all figures. All values are expressed as a mean \pm standard error of the mean, unless otherwise noted.

Results

Table IA displays the mean ± SEM of the pH, Pco₂, Po₂, blood hemoglobin content expressed as oxygen capacity, and oxygen saturation of the maternal arterial and right ventricular blood, neither of which changed significantly as the pregnancy progressed. Except for hemoglobin, these same measurements did change significantly

nificantly for the uterine veins with advancing gestational age (p < 0.001). The mean \pm SEM uterine venous concentrations for gestational days 14 to 30 are shown in Table IB.

Fig. 1, A shows that uterine venous blood pH decreased significantly by approximately 0.1 pH unit as gestation progressed (r = -0.6316, p < 0.001). A major cause of the change in pH was the significant increase in uterine venous Pco2 of approximately 10 torr (Fig. 1, B). There was also a progressive decrease in Po₂ in uterine venous blood from approximately 44 to 30 torr (Fig. 1, C). Oxygen saturation decreased significantly (Fig. 2), thus reflecting the decrease in both pH and Po2 with advancing gestation. The relationship between maternal venous oxygen saturation and PO2 is shown in Fig. 3. The P₅₀ in vivo of the uterine verous blood for the pH range 7.289 to 7.461 was approximately 33 torr, which is in agreement with in vitro data of Battaglia and Barron,6 Jelkmann and Bauer,7 and Dhindsa et al.8

Fig. 2 presents both the arterial and venous oxygen saturations as functions of gestational age. It is clear that the arteriovenous difference in oxygen saturation progressively increases, thus reflecting a greater extraction of oxygen near term. The change in the coefficient of oxygen extraction across the pregnant uterus as a function of gestational age is shown in Fig. 4. The coefficient of extraction reaches a peak value of approximately $60.6\% \pm 0.3\%$ in the last 4 days of gestation.

Since the number of fetuses per horn varied between two and eight, we examined whether the coefficient of extraction changed with the number of fetuses per horn. Fig. 5 presents data obtained between 25 and 30 days' gestation. The coefficient of extraction of oxygen did not vary with the number of fetuses per horn (r = 0.144). Similarly, when animals with other gestational days were compared, no correlation between the number of fetuses and the coefficient of oxygen extraction could be found.

Fig. 6 compares perfusion of the whole body to perfusion of the uterus, both normalized for the oxygen requirement. This was done by comparing the arteriovenous difference in oxygen across the uterus to the arteriovenous difference in oxygen across the whole body. If the uterus and whole body perfusion rate were the same with regard to oxygen consumption, the ratio would be 1. It is apparent that, after approximately 19 days, the arteriovenous difference in oxygen was greater across the uterus than across the whole body. The mean oxygen extraction coefficient for the whole body was $31.3\% \pm 1.5\%$.

Comment

The present study demonstrates that as pregnancy progresses there is a marked increase in oxygen ex-

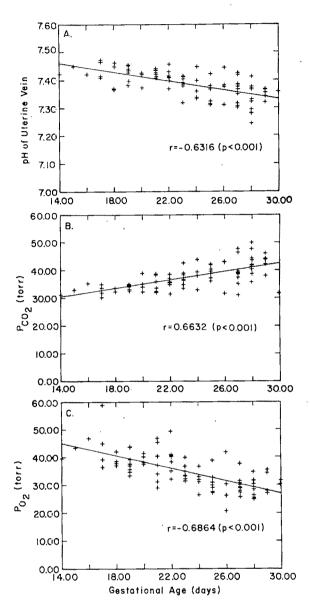


Fig. 1. The data for uterine venous pH, Pco₂, and Po₂ for all animals are plotted versus gestational age. Each point represents the mean of the four arteriovenous differences on the day of study. Linear regression analyses were carried out for each variable and plotted as a solid line with the r and p values included.

traction across the pregnant uterus of the rabbit. The increase in extraction is concurrent with a decline in Po₂ and pH and an increase in Pco₂. In the last 4 days of gestation, the coefficient of extraction of oxygen is $60.6\% \pm 0.3\%$. Litter size has no appreciable effect on the coefficient of oxygen extraction (Fig. 5). This observation agrees with the observations of Johnson et al.4 and Duncan and Lewis,10 in whose studies total uterine flow was found to be increased with increasing litter size. The coefficient of oxygen extraction across the rabbit uterus in late pregnancy is high (1) in relationship to the coefficient of oxygen extraction across the whole body of the rabbit and (2) in comparison to

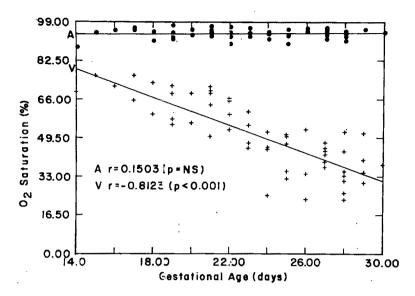


Fig. 2. The maternal arterial (•) and uterine venous (+) saturations are plotted versus gestational age. As in Fig. 1, solid lines depict the calculated linear regressions with the r and p values included.

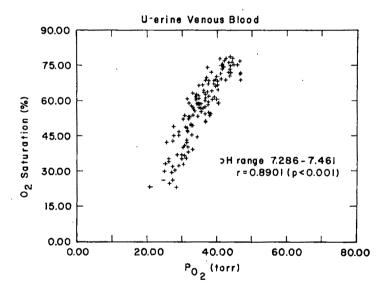


Fig. 3. The in vivo oxygen dissociation curve within the pH range given is depicted for uterine venous blood. For this pH range, the calculated P_{50} in vivo was 32.6.

sheep. Fig. 6 demonstrates that after the twentieth day of gestation, the arteriovenous difference in oxygen is greater across the uterus than across the whole body. Since the reciprocal of the arteriovenous difference in oxygen represents the perfusion required per millimole of oxygen consumption, we can conclude that, as the pregnancy progresses, the rabbit uterus is relatively underperfused in relationship to the rest of the body. Previously, Dhindsa et al.⁸ reviewed data for whole body oxygen extractions and right ventricular Po₂'s. The mean right ventricular Po₂ of 37.8 ± 0.1 (mean ± SEM) found in our study agrees with that of their review of data in several mammalian species. They quoted work by others⁹ that showed an oxygen content differ-

ence across the whole body of the rabbit of $6.2~\rm ml\cdot dl^{-1}$ as compared to $4.1~\rm in$ our data. However, these rabbits were not yet fully grown (mean body weight of $1.9~\rm kg$) and were studied 3 hours after operation and anesthesia, while suspended from a sling. Thus, the studies are not strictly comparable. Our data for whole body oxygen extraction tend to agree with the data of Dhindsa et al. in adult animals which were approximately the same size as our rabbits.

From the viewpoint of comparative physiology, it is important to note that, near the end of pregnancy, the rabbit uterus has a coefficient of oxygen extraction which is approximately 2.5 times higher than that in sheep (Table II). Both species have similar levels of

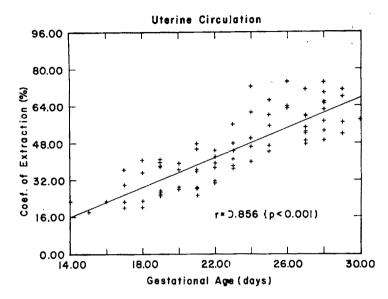


Fig. 4. The calculated coefficient of oxygen extraction across the uterine circulation is plotted versus gestational age. As in previous figures, each symbol represents the mean of the four calculated values on the day of study. The striking increase in coefficient of extraction throughout gestation is evident.

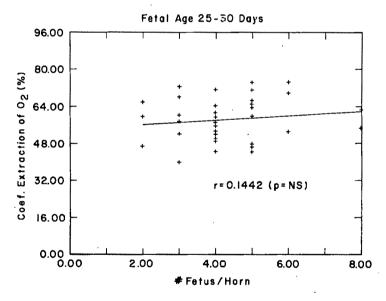


Fig. 5. The coefficient of oxygen extraction is plotted against the number of fetuses per horn for late-gestation animals. There was no relationship between the oxygen extraction and the number of fetuses within the pregnant horn, thus suggesting that flow and fetal mass were proportionately increased as litter size was increased.

blood hemoglobin. Furthermore, the hemoglobin oxygen affinity in rabbits is higher than the hemoglobin oxygen affinity in the genetic type of sheep that provided the comparative data in Table II. As a consequence, the higher oxygen extraction of the rabbit uterus results in a much lower uterine venous Po2 in rabbits (29.3 \pm 0.1 torr) than in sheep (50 \pm 1 torr). We may conclude that, in relationship to oxygen demands, the rate of perfusion of the pregnant rabbit uterus is much less than that in sheep, and that this

difference in perfusion rates cannot be considered to be a compensation for differences in oxygen capacity and/or oxygen affinity. Measurements of placental blood flow by the microsphere technique support this conclusion, by showing that placental blood flow per gram of fetus in rabbits is approximately 2.5 times lower than that in sheep.4

In comparison to sheep, guinea pigs also have low rates of uterine perfusion and a large coefficient of oxygen extraction (Table II). In the absence of the rab-

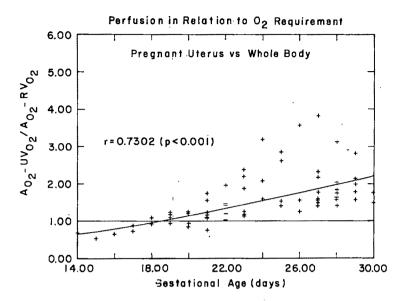


Fig. 6. Comparison of perfusion of the laterus with perfusion of the whole body versus gestational age. The comparison is made as the oxygen content arteriovenous difference across the uterus versus the oxygen content difference between the aorta and the right ventricle. In early gestation (<19 days), the uterus is relatively hyperperfused compared to the rest of the body. However, after 19 days, the ratio exceeds 1, thus implying that the uterus is relatively underperfused compared with the rest of the body.

Table II. Comparative data

	Sheep (130-140 days)	Rabbit (26-30 days)	Guinea pig (50-63 days)
Coefficient of extraction of oxygen (%)	23.4 ± 1.5*	60.6 ± 0.3†	71.7 ± 0.5^{16}
Oxygen saturation (%) (uterine vein)	$67.6 \pm 1.5*$	$38.5 \pm 0.4 \dagger$	26.0 ± 0.5^{16}
Po ₂ (torr) (uterine vein)	$50 \pm 1.0*$	$29.3 \pm 0.1 $ †	
Uterine blood flow (% cardiac output)	15.7 ± 1.3^{12}	6.7 ± 0.06^4	12.8 ± 0.02^{13}
Placental blood flow per gm fetus	0.26 ± 0.02^{12}	0.106 ± 0.008^4	0.114 ± 0.02^{13}
% Fetal/maternal weight	~% ¹²	$7\% \pm 1^4$	$25\% \pm 2^{13}$
Placental type	Venous equi- librator ¹³	Countercurrent exchanger ¹	Countercurrent exchanger ¹⁴

^{*}Unpublished data on seven ewes homozygous for sheep hemoglobin B and carrying a single fetus.

bit data, one might assume that the low rate of perfusion per millimole of oxygen uptake in the guinea pig is due to the large fetal/maternal mass ratio. However, the rabbit has both a low rate of perfusion and a low fetal to maternal weight ratio (Table II). This observation indicates that a low rate of uterine perfusion can be present even in a species that does not develop a large fetal mass. The low placental blood flow of the rabbit and guinea pig in comparison to that of the sneep is probably a reflection of differences in placental type. The rabbit1 and guinea pig14 placentas both are countercurrent exchangers, whereas the sheep placenta is a venous equilibrator.11.15 Thus, the rabbit and guinea pig fetuses can tolerate a lower uterine venous Po2 than the sheep fetus because their mechanism of transplacental oxygen exchange is more efficient than that of the sheep.

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Changes in the distribution of fibronectin in the placenta during normal human pregnancy

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The distribution of fibronectin, a major extracellular glycoprotein with various activities that affect the proliferation and differentiation of cells, was studied by immunofluorescence staining in first-, second- and third-trimester placentas from normal human pregnancy. In early chorionic villi, fibronectin was localized mainly in the trophoblastic basement membranes; this fluorescence became weaker after 10 weeks' gestation. In term placenta, fibronectin was densely deposited around the fetal vessels but not in the trophoblastic basement membranes. Both syncytiotrophoblasts and cytotrophoblasts of the villous epithelium were virtually negative throughout pregnancy. However, the pericellular matrices of nonvillous trophoblasts in early chorionic villi were strongly stained. These findings suggest that fibronectin plays an important role in the proliferation of trophoblastic cells and the tissue organization of the placenta. (AM J OBSTET GYNECOL 1985;152:715-8.)

Key words: Fibronectin, human placenta, trophoblast, basement membrane, immunofluorescence, developmental change

Fibronectin is a high molecular weight glycoprotein with a variety of biologic activities, including cell adhesion, embryonic cell migration, wound healing, and differentiation of cells. ^{1, 2} It is present in soluble form in plasma and amniotic fluid, and in insoluble form in extracellular matrix. The extract of human term placenta has been shown to contain a protein antigenically indistinguishable from plasma fibronectin, ³ and, recently, Zhu et al. ⁴ and we ⁵ have reported the isolation

and biochemical characterization of human placental fibronectin.

The present study was designed to show the distribution of fibronectin in the placenta during normal human pregnancy by indirect immunofluorescence staining. We report here that the distribution of fibronectin changes during the development of chorionic villi.

Material and methods

Placenta. Human trophoblastic tissues were obtained from pregnancies terminated in the first and second trimesters by legal abortion and from normal pregnancies at term immediately after delivery. In the case of term placentas, portions of the central cotyledon were selectively dissected and used for staining. All tissues were examined histologically to assure normal

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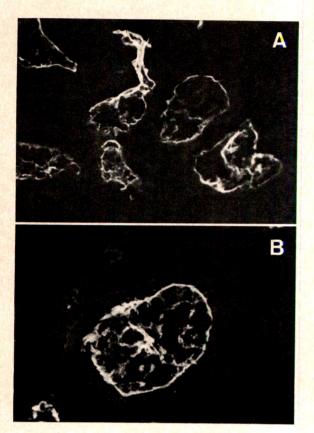


Fig. 1. Chorionic villi of (A) 4 weeks' and (B) 7 weeks' gestation stained by immunofluorescence technique with anti-fibronectin antibodies. Note linear, continuous pattern of fluorescence along the trophoblastic basement membranes. $(A, \times 90; B, \times 180.)$

morphologic features before being immunohistochemically stained. The numbers of placentas studied were 10, 5, and 10 for first, second, and third trimesters, respectively.

Preparation of fibronectin. Human plasma fibronectin was obtained in a pure form from adult human plasma, as described previously.⁶ Human placental fibronectin was isolated according to the method described previously.⁵

Preparation of antibodies. Anti-human fibronectin antibodies were prepared by immunizing rabbits with plasma fibronectin and absorbed with fibronectin-depleted human plasma which had been coupled to Sepharose 4B, as described previously. The antibodies were shown to produce a single precipitin arc when reacted with human plasma by immunoelectrophoresis. Cross-reaction of the antibodies with fibronectin of various sources was tested by the Ouchterlony double immunodiffusion system, as described previously. In this study, we further tested cross-reactivity with collagen types I, III, IV, and V by enzyme-linked immunosorbent assay which was performed as described previously.

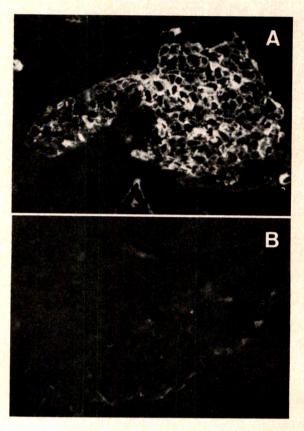


Fig. 2. Cytotrophoblastic cell columns (A) and decidual tissue (B) of 7 weeks' gestation stained with anti-fibronectin antibodies. Note positive staining of nonvillous trophoblasts (A) and virtually negative staining of decidual tissue (B). (\times 180.)

Histologic technique. The distribution of fibronectin was studied by indirect immunofluorescence staining, as described previously. Briefly, unfixed cryostat sections (4 μm) were serially incubated with the anti-fibronectin antibodies and fluorescein isothiocyanate—conjugated goat anti-rabbit immunoglobulin (Behringwerke, Marburg). Each incubation step was carried out at 4° C. Control sections were treated with nonimmune rabbit serum instead of anti-human fibronectin.

Results

Antibodies. The antibodies formed a precipitin line with human placental fibronectin, and the line was completely fused to that of human plasma fibronectin (data not shown). Because of this complete cross-reactivity, we used the antibodies for immunofluorescence staining of placental fibronectin. Enzyme-linked immunosorbent assay showed that the antibodies did not cross-react with any type of collagen tested (data not shown).

Immunofluorescence staining. In the first-trimester chorionic villi, fluorescence of fibronectin was demonstrated in the trophoblastic basement membranes

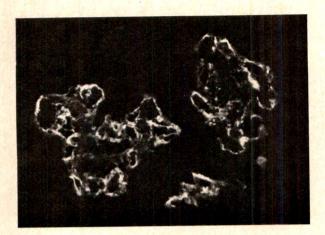


Fig. 3. Chorionic villi of 13 weeks' gestation stained with antifibronectin antibodies. Note that fluorescence in the fetal capillary walls is stronger than that in the trophoblastic basement membranes. (\times 240.)

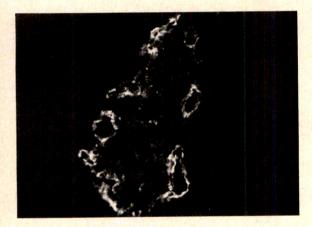


Fig. 4. A chorionic villus of 20 weeks' gestation stained with anti-fibronectin antibodies. Note that fluorescence in the fetal capillary walls is predominant. (×240.)

and in the connective tissue core of the villi (Fig. 1). Fluorescence in the trophoblastic basement membranes was observed in almost all of the villi. The villous stroma showed a loosely organized network of fibrillar fluorescence. Neither syncytiotrophoblasts nor cytotrophoblasts were stained with the antibodies. In contrast, strong fluorescence of fibronectin was found in the pericellular matrices of nonvillous trophoblasts, namely, the cells forming cytotrophoblastic cell columns and cytotrophoblastic shell (Fig. 2, A). Decidual tissue was only faintly stained with the antibodies (Fig. 2, B).

After 10 weeks' gestation, fibronectin was mainly distributed around the fetal capillaries (Fig. 3). Fluorescence in the trophoblastic basement membranes was not so prominent as in the earlier villi. Interruptions of the basement membrane fluorescence were occasionally observed.

In the second-trimester placentas, fibronectin was seen around the fetal vessels and, to a lesser extent, in

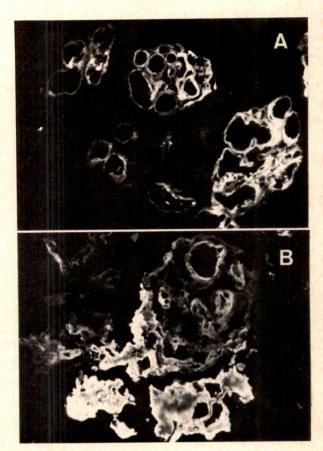


Fig. 5. Chorionic villi of 40 weeks' gestation stained with antifibronectin antibodies (A and B). These specimens were dissected after the perfusion of the placenta with citrated saline solution through the umbilical vein. Note prominent fluorescence in the fetal vessel walls and the absence of fluorescence in the trophoblastic basement membranes (A). The areas of fibrinoid necrosis and intervillous fibrin are strongly positive (B). $(A, \times 300, B, \times 240.)$

the villous stroma (Fig. 4). The trophoblastic basement membranes were no longer recognized as continuous structures.

In term placental villi, fibronectin was stained predominantly around the fetal vessels (Fig. 5). The stainings in the vessel walls were far more dense than those of second-trimester villi. The trophoblastic basement membranes were not stained in almost any of the villi (Fig. 5, A). The syncytiotrophoblast layer was virtually negative, as in the earlier villi. The areas of fibrinoid necrosis and intervillous fibrin were positively stained (Fig. 5, B). In the sections of larger chorionic villi, fluorescence of fibronectin was densely distributed in a laminar fashion around the fetal stem vessels (not shown).

Comment

The present study indicated that both early and term chorionic villi contain fibronectin as a connective tissue component. However, the distribution of fibronectim was different between early and term chorionic vill. In early chorionic villi, fibronectin was localized mainly in the trophoblastic basement membranes. Fluorescence of fibronectin disappeared from the trophoblastic basement membranes with the advance of pregnancy, although studies with the electron microscope have demonstrated that the trophoblastic basement membrane shows an increase in membrane thickness as the placenta matures. Therefore, compositional changes must occur in the trophoblastic basement membrane during the development of chorionic villi.

The present study also revealed that the pericellular matrices of nonvillous trophoblasts of early chorionic villi were stained with anti-fibronectin antibodies, whereas those of villous trophoblasts were not. Boyd and Hamilton⁸ stated that there are numerous miliotic figures in cytotrophoblastic cell columns and cytotrophoblastic shell of early chorionic villi, thus indicating that proliferation is occurring at a high rate. Therefore, the present findings are compatible with the previous observations that actively proliferating undifferentiated cells produce increased amounts of fibronectin. It is likely that trophoblasts have an ability to synthesize fibronectin when they are rapidly proliferating, but lose it as they mature.

In the term placentas, fibronectin was localized around the fetal vessels and in the stroma of the villi. Biochemical characterization of term placental fibronectin recently reported by Zhu et al. and from our laboratory demonstrated that placental fibronectin differs from plasma fibronectin in the carbohydrate structure. This suggests that placental fibronectin is of tissue origin(s) other than plasma. Because of the dense deposition pattern around the fetal vessels, it is unlikely that this fibronectin is of trophoblastic origin. Therefore, term placental fibronectin may be derived from the cells in the connective tissue core, including fibroblasts and endothelial cells; both cell types have been shown to synthesize fibronectin in vitro. 1, 2, 11

The changes in the distribution observed in this study

suggest that placental fibronectin may be produced by different cell types and may play different biologic roles according to the developmental stage of chorionic villi. Fibronectin of early chorionic villi may act not simply as a tissue-supporting material but as a modulator of proliferation and organization of trophoblastic tissues. It would be quite interesting, therefore, to study the effects of fibronectin on the cells of trophectoderm, with the use of cultured animal embryos.

We are grateful to Dr. Hiroshi Nagai for the generous supply of human placentas.

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Erythrocyte filterability and fetal development in normal pregnancy

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The relationship between maternal erythrocyte filterability determined in late pregnancy and infant birth weight was studied in uncomplicated pregnancy. There was a significant positive correlation (r=0.7671, p<0.005). Maternal erythrocyte filterability is suggested to be an indicator of flow state of the placental microcirculation, which affects fetal development. (AM J OBSTET GYNECOL 1985;152:719-20.)

Key words: Birth weight, erythrocytes, erythrocyte filterability, placental microcirculation, rheology

An erythrocyte can pass through vessels smaller than its own diameter. This property of an erythrocyte is referred to as filterability. Reduced erythrocyte filterability has been demonstrated to occur in abnormal pregnancies such as intrauterine growth retardation or severe preeclampsia. In the present study the relationship between filterability of erythrocytes from women with uncomplicated pregnancies and the birth weights of their infants was investigated.

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Material and methods

Forty women with normal pregnancies who were delivered of singleton term infants were selected at random. During 36 to 38 weeks' gestation antecubital venous blood was collected from these subjects and anticoagulated with ethylenediaminetetraacetic acid. Erythrocyte filterability was determined by a filtration method modified by us.² Correlations were performed by means of the method of least squares.

Results and comment

The birth weights of the infants ranged from 2500 to 3980 gm. The erythrocyte filterability ranged from 7.5 to 24.3 μ l/sec. The relationship between them is demonstrated in Fig. 1. There was a highly significant correlation (r = 0.7671, p < 0.005) (Fig. 1).

It has been demonstrated that fetal development is

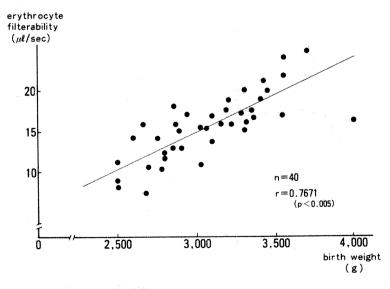


Fig. 1. The relationship between erythrocyte filterability of maternal blood and the birth weights of infants.

dependent on the uteroplacental blood flow. When this flow is reduced, fetal development is impaired. In such circumstance, a decrease in erythrocyte filterability has also been reported. Although the area of erythrocyte filterability and its influence on the placental circulation is still new, it is apparent that the filterability of maternal erythrocytes is closely related to the flow state of the placental microcirculation and that it affects fetal development in normal pregnancy as well as in cemain abnormal pregnancies.

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Conservative surgery for the management of a second-trimester cervical pregnancy

To the Editors:

I would like to take this opportunity to comment on the article by ten Kate-Booij and Wallenburg entitled "Conservative treatment of postpartum hemorrhage in a second-trimester cervical pregnancy" (AM J OBSTET GYNECOL 1984;150:103).

The authors are to be congratulated for their aim of preserving the childbearing function of a young patient who suffered a cervical pregnancy. This must always be the goal of the clinician. However, they must be very lucky because they were able to control bleeding by packing the cervical cavity and by abdominal compression.

From the literature available to me concerning cervical pregnancy during the second trimester, only in one case (Lange and Sjoolie) were they able to control hemorrhage in a 22-year-old patient by curettage and cervical packing.

In a more recent publication⁵ conservative surgery was proposed if other conservative measures failed to control the massive bleeding.

It is suggested that after hysterotomy, by partial obliteration of the enlarged cervical cavity with interrupted sutures, as after the enucleation of large cervical myoma, hysterectomy can be avoided.

In this respect the publication of ten Kate-Booij and Wallenburg is not the first one that describes successful conservative treatment of a cervical pregnancy discovered in the second trimester.

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Reply

To the Editors:

We are pleased to comment on Dr. Tympanidis' letter. The essence lies in the final remark that we were not the first to describe successful conservative treatment of postpartum hemorrhage in a second-trimester cervical pregnancy. We were aware of the publication by Lange and Sjoolie (1975), but in the case described by these authors there was only minimal bleeding. Furthermore, the authors themselves cast doubt on the diagnosis of cervical pregnancy because histologic examination showed chorionic villi in tissue removed from the cervical canal as well as in that obtained from the uterine cavity. Dr. Tympanidis' 1984 article is certainly of considerable interest, but it was published at the same time as ours and could therefore not be known to us when we prepared our report.

Finally, we may have been luckier than others, but we would like to emphasize that, as demonstrated in the literature cited by Dr. Tympanidis, packing alone will usually not be sufficient to control the bleeding. The essential procedure is compression of the bleeding cervix between the abdominal hand and the intravaginal hand for an extended period of time.

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Fetal pericardial fluid

To the Editors:

One of the conclusions of Jeanty et al. in their article entitled "Fetal pericardial fluid: a normal finding of the second half of gestation" (AM J OBSTET GYNECOL 1984;149:529) is that the layer of pericardial fluid never exceeds 2 mm in patients between 26 and 40 weeks of gestation. If it does exceed 2 mm, further investigations should be performed to exclude reactive hyperemia,

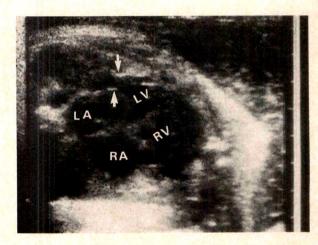


Fig. 1. Four-chamber apical view. The 4 mm space between the *arrows* is the pericardial fluid layer. *LA*, left atrium; *LV*, left ventricle; *RA*, right atrium; *RV*, right ventricle. (Aloka SSD-256.)

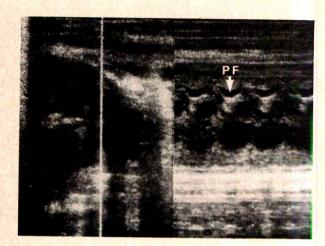


Fig. 2. M-mode directed two-dimensional echocardiography. The cursor is at the level of the arrows in Fig. 1. A clear-cut pericardial fluid *(PF)* layer is demonstrated *(arrow)*.

hypoalbuminemia, immune disease, and other causes of hydrops fetalis.

Just recently we performed an ultrasound examination of a woman at 39 weeks of gestation to rule out intrauterine growth retardation. On two-dimensional real-time and M-mode ultrasound we visualized a considerable amount of pericardial fluid. The width of the pericardial fluid layer was 4 to 5 mm (Figs. 1 and 2). Cardiac anatomy and function were normal. A healthy normal male infant was delivered a week later.

Three days after delivery two-dimensional real-time and M-mode echocardiography confirmed the diagnosis of pericardial effusion. The effusion disappeared gradually during the following week. A thorough investigation revealed a completely healthy neonate.

We wonder whether the normal range of pericardial fluid width may be larger than that proposed by Jeanty et al.

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Dorsiflexion of the big toe—A sign of impending fetal micturition

To the Editors:

It is well known to pediatric radiologists performing voiding cystoureterograms that pseudo–Babinski sign heralds micturition in young infants. This sign is not well understood but probably relates to an as yet non-mature central nervous system.

We observed the same phenomenon occurring in utero in almost every case (Fig. 1). Whenever dorsiflexion of the big toe is observed by ultrasound, immediate attention should be focused to the fetal urinary bladder. Indeed the bladder size diminishes, and its



Fig. 1. Fetal ultrasound demonstrating dorsiflexion of the big toe (arrow indicates big toe).

image disappears. The importance of this observation is that it enables the sonographer to study fetal micturition. It is quite possible that such diagnoses as primary vesicoureteral reflux and posterior urethral valve could be precisely identified in utero.

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Prophylactic antibiotics unjustified for unselected abortion patients

To the Editors:

Grimes and co-authors are esteemed colleagues whose analysis of prophylactic antibiotics for curettage

abortion (AM J OBSTET GYNECOL 1984;150:689-94) unfortunately omits or leaves unclarified a number of important aspects and is misleading. Although the authors do not come to any conclusion other than "the literature suggests that prophylaxis is effective in reducing febrile morbidity from abortion," they, and a number of readers, unjustifiably conclude that antimicrobial chemoprophylaxis is universally required for this procedure and that the primary question remaining is determination of the "optimal antibiotic regimen." This is not the case.

The three randomized clinical trials analyzed and another larger, unpublished, double-blinded, placebocontrolled, randomized study do not establish the benefit of uniform chemoprophylaxis for curettage abortion.1-4 Stewart's study of approximately 2000 firsttrimester curettage abortions in private patients demonstrates no benefit from receiving tetracycline versus placebo in preventing postabortal pelvic infection.4 The 95% confidence limits of relative risk for outcomes in the studies of Krohn and Westrom and their colleagues include 1.0 and do not demonstrate statistical significance.1.2 The third and largest analyzed study performed by Sonne-Holme et al.3 does demonstrate statistical significance. Sonne-Holme et al. usefully point out that the subgroup of women with a clinical history of pelvic inflammatory disease accounted for this difference. These authors conclude that "prophylactic administration of antibiotics for first-trimester abortion should be used in women who have had pelvic inflammatory disease." Studies of the related question of chemoprophylaxis for hysterosalpingography yield similar results and recommendations.5,6 Other studies analyzing the cervical presence of upper genital tract pathogens Chlamydia trachomatis and Streptococcus agalactiae on first-trimester postabortal morbidity do not demonstrate excess risk.7

The risk/benefit ratio of tetracycline use for induced abortion is more complex than is considered. The evaluation of Harrison et al. of the effect of a short course of prophylactic minocycline on acquisition of gonorrhea demonstrated that this treatment was not only incompletely effective but was associated with selection of relatively resistant strains of *Neisseria gonorrhoeae*. Other untoward reactions to tetracycline and other antibiotic use include hypersensitivity, photosensitivity, immunosuppression, increased susceptibility to enteric infection, candidiasis, and ecologic alteration. Recent data demonstrate human infection with antibiotic resistant bacteria ascribed to routine use of tetracycline in the environment. 12

The authors equate the sequelae of postabortal febrile morbidity, which is most frequently endometritis, with community-acquired acute salpingitis and suggest substantially increased risks of infertility and ectopic pregnancy. A recent review by Hogue et al.¹³ does not establish these risks. Neither does a recent evaluation of postcesarean febrile morbidity caused presumably by endometritis.¹⁴ The evaluation of pelvic infection

by Svensson et al.¹⁵ confirms the differing fertility outcomes of presumed endometritis and salpingitis. Chung et al.¹⁶ note that the highest rates of postabortal ectopic pregnancy occur in women with retained secundines. This observation emphasizes the importance of operative care in preventing postoperative complications. Certainly all postabortal morbidity should be treated expeditiously and effectively.

In sum, present data do not establish that antibiotic prophylaxis in first-trimester abortion is of benefit to all groups of women. Rational chemoprophylaxis requires delineation of patients who are at measurable risk and who may measurably benefit from a specific treatment. In the absence of further satisfactorily controlled evaluations, Sonne-Holme et al.'s recommendation of short-course antibiotic prophylaxis restricted to women with a clinical history of pelvic inflammatory disease appears appropriate. As noted, microbiologic identification of N. gonorrhoeae and C. trachomatis lower genital tract infection with full treatment of patients and partners with test of cure is required. Indiscriminate chemoprophylaxis may lead to inadequate therapy, hasten appearance or selection of resistant bacteria, and be a source of care provider apathy in using aseptic technique and in screening and treatment of gonorrhea and chlamydia infections. Recommendation of antimicrobial chemoprophylaxis for unselected women requiring first-trimester abortion remains unjustified. Such recommendations are reminiscent of the cartoon in The New Yorker in which an arms dealer says to a prospective customer, "What we are selling here is peace of mind"-rather than a proven benefit.

James A. McGregor, M.D.C.M.

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Reply

To the Editors:

We appreciate the opportunity to respond to Dr. McGregor's thoughtful letter. His comments show how even the closest of colleagues can disagree when interpreting the scientific literature. We will address his concerns in sequence.

First, we believe that data summarized in our report do "establish the benefit" of routine prophylaxis. We were familiar with the oral presentation of Dr. Stewart. but we chose not to include it in our review, since it has not been completely analyzed, subjected to peer review, or published. Of more importance, it is the only study not to have found a reduction in morbidity associated with prophylactic antibiotics. On the other hand, a recent randomized clinical trial of metronidazole for first-trimester abortion has found a powerful protective effect of prophylaxis. The morbidity in the treated group was reduced by four-fifths (p < 0.025). Another study² has found a powerful protective effect of prophylactic antibiotics. This linear logistic regression analysis of 26,332 first-trimester abortions found that use of prophylactic antibiotics reduced febrile morbidity by two-thirds, a statistically significant decrease. Publication of this report will bring to 10 the total number of reports in the literature, all of which have found a protective effect of prophylaxis.

The fact that not all the decreases in morbidity reported in our review reached statistical significance may be the function of small-sample sizes; this limitation is more than overcome by the striking consistency of the protective effect found by investigators around the world. McGregor also cites one unpublished study suggesting that the presence of *Chlamydia trachomatis* in the cervix does not increase the risk of postabortal mor-

bidity. Again, the weight of published evidence³⁻⁵ suggests otherwise (including one study⁶ published in the same issue as our article).

Second, Dr. McGregor notes that administration of tetracycline is not without risks. We agree. Nevertheless, serious complications are extremely rare. As noted by the American Medical Association, "All tetracyclines have relatively low toxicity at usual dose levels." Moreover, one group of infectious disease experts8 has stated that "the activity of doxycycline against aerobic gramnegative organisms, as well as Bacteroides fragilis, combined with its ability to penetrate tissues of the female reproductive system and gastrointestinal tract, and its 12-hour dosing interval make it an ideal agent for prophylaxis in vaginal and abdominal hysterectomies and gastrointestinal surgery." The risk of such short-term therapy leading to antibiotic resistance is extremely slight; giving prophylaxis for abortion is not analogous to adding antibiotics to cattle feed.9

Third, we agree with McGregor that endometritis is a more frequent infectious complication of abortion than is salpingitis, yet the risk of the latter is well established. Prevention of both endometritis and salpingitis is the goal of prophylaxis. Whether salpingitis is acquired through sexual contacts in the community or through curettage abortion seems irrelevant; salpingitis damages subsequent fertility. In addition, while abortions in general are not associated with an increased risk of ectopic pregnancy in later pregnancies, those abortions complicated by infection may be. The overall lack of association between induced abortion and both infertility and ectopic pregnancy may reflect the current widespread use of prophylactic antibiotics.

Fourth, Dr. McGregor notes that the "present data do not establish that antibiotic prophylaxis in first-trimester abortion is of benefit to all groups of women." Again, we agree. As we discussed in our article, selective administration of prophylaxis to those women at highest risk of infection would be desirable. Unfortunately, we feel current methods of identifying those high-risk groups are inadequate. Since all published studies have shown a lowering of overall morbidity when prophylaxis is used, routine use seems a preferable alternative until better methods for identifying high-risk women are available. We feel the next clinical trials of antibiotic prophylaxis should address this issue.

The Medical Letter consultants¹¹ cited only two of the eight published studies reviewed in our article. Since these consultants may have been unaware of the majority of studies which we had on this subject, it is understandable that we reached somewhat different conclusions. Although McGregor states that the Medical Letter "does not recommend antimicrobial administration for uterine curettage," the issue in question lists under "Recommended Drugs" aqueous penicillin G for first-trimester abortion patients with previous pelvic inflammatory disease and cefazolin for second-trimester abortion patients.

Prophylactic antibiotics are widely used for many

types of surgery in the United States.¹² Use of short-term prophylactic antibiotics for curettage abortion likewise is firmly entrenched in abortion practice in the United States; a 1981 survey of members of the National Abortion Federation (who provide about half of all abortions in this country) revealed that "70% always or usually prescribe antibiotics following the procedure." Thus we estimate that at least several million women have received prophylactic antibiotics for abortion during the past dozen years in the United States. We are unaware of any serious complications from adverse reactions to these antibiotics. On the other hand, infection is a leading cause of death from abortion.

Use of tetracyclines for prophylaxis in this setting is inexpensive, safe, and effective. We believe the benefits of prophylaxis outweigh the risks. In the absence of better methods of identifying women at high risk, routine use seems reasonable on the basis of existing information. As noted by the arms dealer in McGregor's New Yorker cartoon, we indeed hope to be promoting peace of mind—and we are sticking to our guns.

David A. Grimes, M.D. Kenneth F. Schulz, M.B.A. Willard Cates, Jr., M.D., M.P.H.

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Total sulfhydryl compounds in human seminal plasma

To the Editors:

Thiols are critical for regulation of enzymatic activity, protein synthesis, membrane transport systems, microtubule assembly, hormone structure and receptors, conformation and aggregation of proteins. Human semen is rich in proteins, enzymes, and spermatozoa. Maintenance of structure and function of spermatozoa, spermatozoal motility, possibly capacitation, and even fertilization may similarly be dependent upon such thiol compounds. It has been shown that spermicidal compounds like copper exert their action by binding sulfhydryl groups.2 As there was very scant information available regarding the total sulfhydryl compounds in human seminal plasma, estimation of total sulfhydryl compounds by use of the method of Beutler et al.3 was carried out. Human semen collected by masturbation after a period of 5 days of abstinence from normal volunteers (n = 15; 25 ± 5 years old) and patients with prostatic infection (n = 15; 30 ± 5 years old) were subjected to routine semen analyses (Table I) along with estimations of fructose and zinc as markers of accessory reproductive glandular functions.4

As reported earlier there was marked decrease in sperm density, motility, semen volume, and biochemical markers studied in the semen of patients with prostatic

Table I. Seminal parameters studied*

Parameter	Controls	Patients	
 Semen volume (ml)	3.0 ± 1.0	1.5 ± 0.25†	
На	7.45 ± 0.05	$7.95 \pm 0.075 \dagger$	
Sperm density (1000/ml)	80.00 ± 15.00	$28.00 \pm 5.5 \dagger$	
Sperm motility (%)	70.00 ± 5.00	$30.00 \pm 6.5 \dagger$	
Fructose (mmol/L)	9.80 ± 1.25	10.50 ± 2.00	
Zinc (mmol/L)	2.20 ± 0.75	$0.60 \pm 0.40 \dagger$	
Total sulfhydryl compounds	165.00 ± 25.00	$90.00 \pm 25.00 \dagger$	
(mg/dl)			

^{*}Values are means ± SEM of 15 cases per group.

tp = 0.01.

infection.⁵ Along with the fall in these parametem the levels of total sulfhydryl compounds were also comparatively lower in the patients' samples. There seems to be good amounts of sulfhydryl compounds present in the seminal plasma of normal cases. Thus it appears worthwhile to study further the role of thiols in seemen metabolism.

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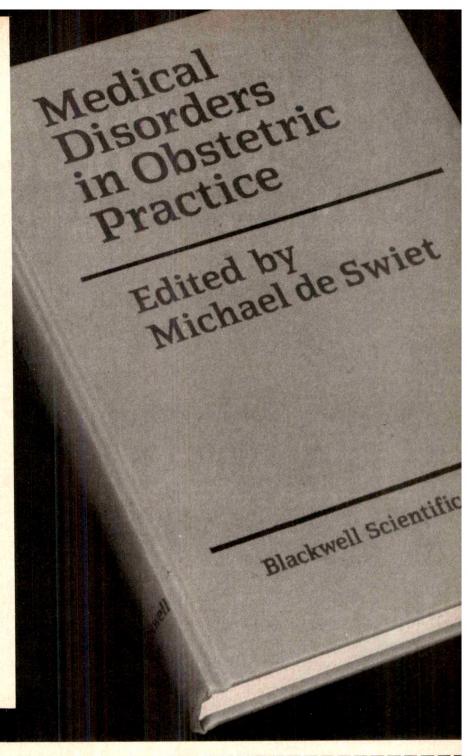
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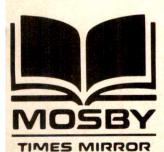
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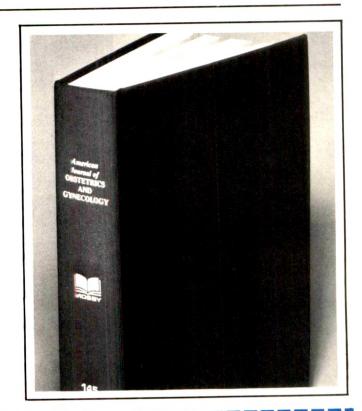
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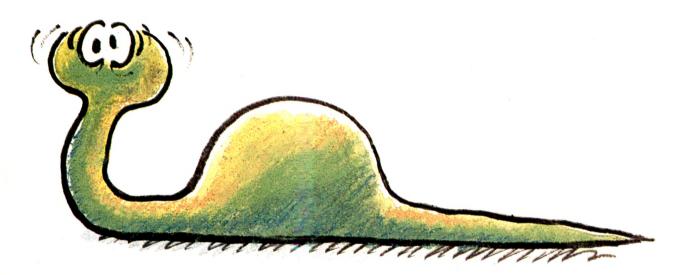
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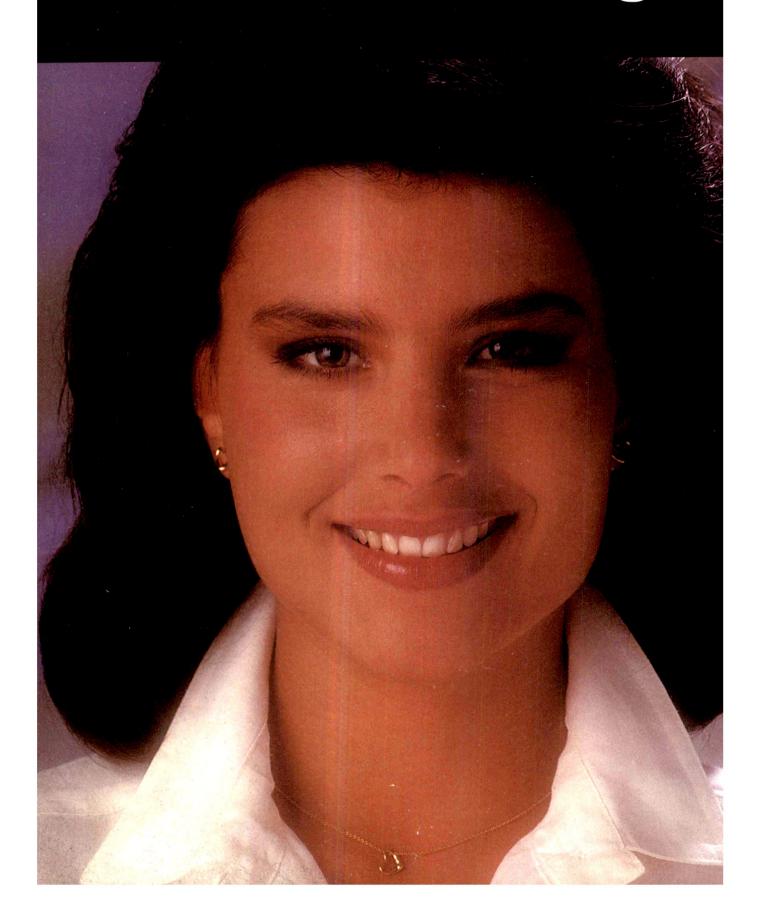
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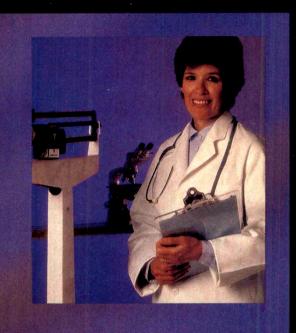
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ORAL CONTRACEPTIVE (O.C.) AGENTS

ORAL CONTRACEPTIVE (O.C.) AGENTS
Indications Prevention of pregnancy DOSE-RELATED RISK OF THROMBOEMBOLISM. Because studies have shown a positive association between OC estrogen dose and risk of thromboemboilsm. It is prudent to minimize estrogen
exposure. Prescribe an OC with the least amount of estrogen compatible with an
acceptable pregnancy rate and patient acceptance. Start new users on OCs
containing OS mg or less of estrogen.
Ceatra-indications 1. Known or suspected pregnancy (see Warning #5). 2.
Thromboehbeithis or thromboemboilc disorders. 3. Past history of deep ven
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thromboehbeithis or thromboemboilc disorders. 4. Undiagnosed abnormal gentla
beeding. 5. Ocs should not be used by women who have or have had any of the
following: a. cerebral vascular or coronary arriery disease, including myocarda
inflanction. b. known or suspected carcinoma of the breast. c. known or suspected estrogen dependent negolissa. d. benign or malignant liver tumor that
developed during used of Ocs or other estrogen containing products.

WARNINGS: Cigarette smoking increases the risk of serious cardiovascu-lar side effects from OC use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use OCs should be strongly advised not to smoke.

to smote.

The use of OCs is associated with increased risk of several serious conditions including thromboembolism, stroke, myocardial infarction, liver tumor, gall bladder disease, visual disturbances, fetal abnormalities, and hypertension. Practitioners prescribing OCs should be familiar with the following information retating to these risks.

Practitioners prescribing ULS snould be raminar with the nonlowing information relating to these risks.

1. Thromboembolic Disorders and Other Vascular Problems: An increased risk of thromboembolic and thrombotic disease associated with OU use is established no study demonstrated an increased relative risk for itaal venous thromboembolism and several studies demonstrated if for non-fatal venous thromboembolism. They estimate that OC users are 4-11 times more likely than nonusers to develop these diseases without evident cause. One British study propried an excess death rate of 40% in OC users most of which resulted from cardiovascular disease. Another British study showed a lower death rate in OC users the controls; an increase in cardiovascular disease was seen but was not statistically significant. A U.S. prospective study failed to disclose increased mortality rates from cardiovascular disorders in some significant increases in venous thromboembolism. CERT-storm cardiovascular disorders, but a subset analyzed as a retrospective, case-control study showed significant increases in venous thromboembolism. CERT-storm cardiovascular disorders in women with and without predisposing causes, relative risk for stroke not shown in prior British studies. In an American study and thrombotic stroke as 4-45 times greater in users than nonusers. A British long-term follow-up study reported in 1976 a highly significant association between the properties of the properties of the stroke and the study had suggested an association in 1974, but the number of cases was too small to estimate the risk. Subarachnoid hemorrhagic smoking alone increases in know than either alone in British and American studies. Smoking alone increases in know the lane either alone in British and American studies. Smoking alone increases in know the han either alone in British and American studies.

number of cases was too small (n estimate the risk Subarachnoid hemorrhage has been shown to be increased by OC use in British and American studies. Smoking alone increases in incidence of these accidents; smoking and pill use appear to increase risk more than either alone.

MYOCARDIAL INFARCTION (MI). Increased relative risk of MI associated with OC use has been reported. One British study found that the greater the number of the control of the properties of the properties of the control of the control of the properties of the control of t

CONDITIONS—RISK ASSOCIATED WITH USE OF OCS					
Age	Below 30	30-39	40 +		
Heavy smokers Light smokers Nonsmokers	C D	B C	A B		
(no predisposing conditions) Nonsmokers	D	C,D	C		
(other predisposing conditions)	С	C.B	B.A		

Physician and patient strouble be alert to earliest manifestations of thromboemboria and thrombotic disorders (e.g., inconnecipitedis, pulmonary embolism, cerebility). The control of the property of of the

clinical significance is unknown. 8. Elevated Blood Pressure: An increase in blood pressure has been reported with Ouse hypertension may occur within a lew months of posigning OS: In the first year of use, incidence on typertension may be no higher in OC users than in nonusers; incidence in users increases with some than the properties of the pressure of the press





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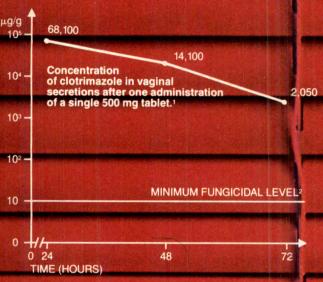
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PRECAUTIONS: If there is a lack of response to Mycelex 8-G 500 mg Vaginal Tablets, appropriate microbiological studies should be repeated to confirm the diagnosis and rule out other pathogens before instituting another course of anti-mycotic therapy.

mycotic interapy.

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USABE IN PREGNANCY: PREGNANCY CATEGORY B: The disposition of 14C-clotrimazole has been studied in humans and animals. Clotrimazole is poorly because the studies of the contraction of the studies of the studi

absorbed following intravaginal administration to humans, whereas it is rather well absorbed after oral administration.

ausurbez tolowing intravaginal administration. In clinical trials, use of vaginally applied clotrimazole in pregnant women in their second and third trimesters has not been associated with ill effects. There are, however, no adequate and well-controlled studies in pregnant women during the first trimester of pregnancy. Studies in pregnant regnancy studies in pregnant regnancy studies in pregnant regnancy are revealed no evidence of harm to the fetus due to clotrimazole. Repeated high oral doses of clotrimazole in rats and mice ranging from 50 to 120 mg/kg resulted in embryotoxicity (possibly secondary to maternal toxicity), impairment of mating, decreased plura size and number of viable young accreased liters are and unimber of viable young accreased pure size and number of viable young accreased pure produced to the produced pro

dose.

Because animal reproduction studies are not always predictive of human response, this drug should be used only if clearly indicated during the first

response, this trulg situation be used only in clearly indicated using the miss trimester of pregnancy.

ADVERSE REACTIONS: 01 297 patients in double-blind studies with the 500 mg vaginal tablet, 3 of 149 patients treated with active drug and 3 of 149 patients treated with placebo reported complaints during therapy that were possibly drug related. In the active drug group, vomiting occurred in one patient, vaginal soreness with cottus in another, and complaints of vaginal irritation, ichting, burning and dyspareunia in the third patient. In the placebo group, clitoral irritation occurred in one patient and dysura, described as remotely related to drug, in the other. A third patient in the placebo group developed bacterial vaginitis which the investigator classed as possibly related to drug.

Eighteen (1 8/3) of the 1115 patients treated with Mycelex®-G 500 mg in other formulations in double-blind studies reported complaints during therapy that were possibly drug-related. Mild burning occurred in six patients while other complaints such as skin rash, (ching, vulval irritation, lower abdominal cramps and bloating, slight cramping, slight urinary frequency, and burning or irritation in the sexual partner, occurred rarely.

OVERDOSABE: No data available.

OVERDOSAGE: No data available

DRUG ABUSE AND DEPENDENCE: Drug abuse and dependence with Mycelex®-G 500 mg Vaginal Tablets has not been reported.

DOSAGE AND ADMINISTRATION: The recommended dose is one tablet inserted DOSAGE AND ADMINISTRATION: The recommended dose is one tablet inserted intravaginally one time only, preferably at bedtime. In the event of treatment failure, that is, persistence of signs and symptoms of vaginitis after five days, other pathogens commonly responsible for vaginitis should be ruled out before instituting another course of antimycotic therapy.

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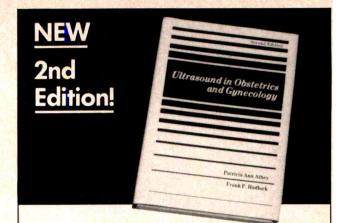
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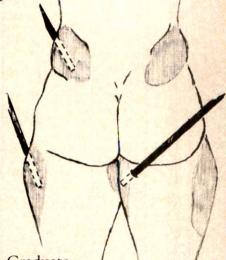
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Morphine sulfate may be habit forming. (See Drug Abuse and Dependence section.)

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PRECAUTIONS
GENERAL
Preservative-free DURAMORPH® PF (Morphine Sulfate Injection, USP) should be administered with extreme caution in aged or debilitated patients, in the presence of increased infracranial/intraocularpressure and in patients with head injury. Pupillary changes (miosis) may obscure the course of intracranial pathology. Care is urged in patients who have a decreased respiratory reserve (e.g., emphysema, severe obesity, kyphoscoliosis). Seizures may result from high doses. Patients with known seizure disorders should be carefully observed for evidence of morphine-induced seizure activity.

It is recommended that administration of DURAMORPH® PF by the epidural or intrathecal routes be limited to the lumbar area. Intrathecal use has been associated with a higher incidence of respiratory depression than epidural use.

Smooth muscle hypertonicity may result in biliary colic, difficulty in urination and possible urinary retention requiring catheterization. Consideration should be given to risks inherent in urethral catheterization, e.g., sepsis, when epidural or intrathecal administration is considered, especially in the perioperative period.

Elimination half-life may be prolonged in patients with reduced metabolic rates and with hepatic or renal dysfunction. Hence, care should be exercised in administering morphine in these conditions, particularly with repeated dosing.

Patients with reduced circulating blood volume, impaired myocar-dial function or on sympatholytic drugs should be observed care-fully for orthostatic hypotension, particularly in transport.

Patients with chronic obstructive pulmonary disease and patients with acute asthmatic attack may develop acute respiratory failure with administration of morphine. Use in these patients should be reserved for those whose conditions require endotracheal intubation and respiratory support or control of ventilation.

DRUG INTERACTIONS

DRUG INTERACTIONS
Depressant effects of morphine are potentiated by either concomitant administration or in the presence of other CNS depressants such as alcohol, sedatives, antihistaminics or psychotropic drugs (e.g., MAO inhibitors, phenothiazines, butyrophenones and tricyclic antidepressants). Premedication or intra-anesthetic use of neuroleptics with morphine may increase the risk of respiratory depression.

CARCINOGENESIS, MUTAGENESIS, IMPAIRMENT OF FERTILITY Studies of morphine sulfate in animals to evaluate the carcinogenic and mutagenic potential or the effect on fertility have not been conducted.

PREGNANCY

PRESIANCY
Teratogenic effects—Pregnancy Category C. Animal reproduction studies have not been conducted with morphine sulfate. It is also not known whether morphine sulfate can cause fetal harm when administered to a pregnant woman or can affect reproduction capacity. Morphine sulfate should be given to a pregnant woman only if clearly needed.

Nonteratogenic effects. Infants born from mothers who have been taking morphine chronically may exhibit withdrawal symptoms.

taking morphine chronically may exhibit withdrawal symptoms. LABOR AND DELIVERY Intravenous morphine readily passes into the fetal circulation and may result in respiratory depression in the neonate. Naloxone and resuscitative equipment should be available for reversal of narcotic-induced respiratory depression in the neonate. In addition, intravenous morphine may reduce the strength, duration and frequency of uterine contracting resulting in prolonged labor. Epidurally and intrathecally administered morphine readily passes into the fetal circulation and may result in respiratory depression of

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However, studies have suggested that in most cases 0.2 to 1 mg of morphine intrathecally provides adequate pain relief with little effect on the duration of first stage labor. The second stage labor, though, may be prolonged if the parturient is not encouraged to bear down. A continuous intravenous infusion of naloxone. 0.6 mg/hr, for 24 hours after intrathecal injection may be employed to reduce the incidence of potential side effects.

NURSING MOTHERS

Morphine is excreted in maternal milk. Effect on the nursing infant is not known.

PEDIATRIC USE Safety and effectiveness in children have not been established.

Safety and effectiveness in children have not been established.

ADVERSE REACTIONS

The most serious side effect is respiratory depression. Because of delay in maximum CNS effect with intravenously administered drug (30 min), rapid administration may result in overdosing. Bolius administration by the epidural or intrathecal route may result in early respiratory depression due to direct venous redistribution of morphine to the respiratory centers in: the brain. Late (up to 24 hours) onset of acute respiratory depression has been reported with administration by the epidural or intrathecal route and is believed to be the result of rostral spread. Reports of respiratory depression following intrathecal administration have been more frequent, but the dosage used in most of these cases has been considerably higher than that recommended. This depression may be severe and could require intervention (See Warnings and Overdosage, sections). Even without clinical evidence of ventilatory inadequacy, a diminished CO₂ ventilation response may be noted for up to 22 hours following epidural or intrathecal administration.

While low doses of intravenously administered morphine have little

While low doses of intravenously administered morphine have little effect on cardiovascular stability, high doses are excitatory, resulting from sympathetic hyperactivity and increase in circulating catecholamines. Excitation of the central nervous system resulting in convulsions may accompany high doses of morphine given intravenously. Dysphoric reactions may occur and toxic psychoses have been reported.

Epidural or intrathecal administration is accompanied by a high incidence of pruritus which is dose related but not confined to site of administration. Nausea and vomiting are frequently seen in patients following morphine administration. Urinary retention which may persist for 10-20 hours following single epidural or intrathecal administration has been reported in approximately 90% of males. Incidence is somewhat lower in females. Patients may require catheterization (see Precautions) Pruritus, nausea/vomiting and urinary retention frequently can be alleviated by the intravenous administration of low doses of naloxone (0.2 mg).

Tolerance and dependence to chronically administered morphine, by whatever route, is known to occur (see Drug Abuse and Dependence section).

Miscellaneous side effects include constipation, headache, anxiety, depression of cough reflex, interference with thermal regulation and oliguria. Evidence of histamine release such as uticaria, wheals and/or local tissue irritation may occur.

and/or local issue inflation in any occur.

In general, side effects are amenable to reversal by narcotic antagonists. NALOXONE INJECTION AND RESUSCITATIVE EQUIPMENT SHOULD BE IMMEDIATELY AVAILABLE FOR ADMINISTRATION IN CASE OF LIFE-THREATENING OR INTOLERABLE SIDE EFFECTS.

DRUG ABUSE AND DEPENDENCE Controlled Substance: Morphine sulfate is a Schedule II substance under the Drug Enforcement Administration classification

Abuse: Morphine has recognized abuse potential.

Dependence: Cerebral and spinal receptors may develop toler-ance/dependence independently, as a function of local dosage. Care must be taken to avert withdrawal in those patients who have been maintained on parenteral/oral narcotics when epidural or intrathecal administration is considered. Withdrawal may occur following chronic epidural or intrathecal administration, as well as the development of tolerance to morphine by these routes. (See Nonteratogenic effects under Pregnancy.)

Overdosage is characterized by respiratory depression with or without concomitant CNS depression. Since respiratory arrest may result either through direct depression of the respiratory center or

as the result of hypoxia, primary attention should be given to the establishment of adequate respiratory exchange through provision of a patent airway and institution of assisted or controlled ventilation. The narcotic antagonist, naloxone, is a specific antidote. Naloxone (usually 0.4 mg) should be administered intravenously, simultaneously with respiratory resuscitation. As the duration of effect of naloxone is considerably shorter than that of epidural or intrathecal morphine, repeated administration may be necessary. Patients should be closely observed for evidence of renarcotization. Note: Respiratory depression may be delayed in onset up to 24 hours following epidural or intrathecal administration. In painful conditions, reversal of narcotic effect may result in acute onset of pain and release of catecholamines. Careful administration of naloxone may permit reversal of side effects without affecting analgesia. Parenteral administration of narcotics in patients receiving epidural or intrathecal morphine may result in overdosage.

HOW SUPPLIED

Amber Dosette® ampuls for intravenous, epidural and intrathecal administration.

5 mg/10 mL (0.5 mg/mL) packaged in 10s (NDC 0641-1113-33) 10 mg/10 mL (1 mg/1 mL) packaged in 10s (NDC 0641-1115-33) Revised September 1984

- Cohen SE, Woods WA. Anesth 58:500, 1983
- Rawal N, Sjöstrand U, Christoffersson E, et al. Anesth Analg 63:583, 1984



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September 7-8

ommon Problems in Reproductive Endocrinology"

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November 2-3

"Update in Maternal-Fetal Medicine"

Frank C. Miller, M.D. Jerry Wiley, J.D. Lawrence Platt, M.D. Julian P. Parer, M.D.

COMMON PROBLEMS IN REPRODUCTIVE ENDOCRINOLOGY

Saturday, September 7, 1985

7:30 AM Alan DeCherney, M.D.

"In Vitro Fertilization" 8:30 AM Charles March, M.D.

"What's New with GnRh"

9:30 AM Alan DeCherney, M.D.

"Ectopic Pregnancy"

10:30 AM Jorge Mestman, M.D.

"Evaluation and Management of Thyroid

Disorders in Pregnancy"

11:30 AM Alan DeCherney, M.D. "Infertility Surgery"

Sunday, September 8, 1985

7:30 AM Charles March, M.D.

"Current Status of Hysteroscopy in Gynecology"

8:30 AM Sergio Stone, M.D.

"The Evaluation and Work-up of the Patient

With Excess Androgen Production"

9:30 AM Charles March, M.D.

"Current Status of Ultra Sound in Gynecology"

10:30 AM Sergio Stone, M.D.

"A Re-evaluation of the Cervical

Factor in Infertility"

11:30 AM Jorge Mestman, M.D.

"Multiple Endocrine Deficiency Syndrome"

UPDATE IN MATERNAL-FETAL MEDICINE

Saturday, November 2, 1985

7:30 AM Julian P. Parer, M.D.

fanagement of Abnormal Heart Rate Patterns

During Pregnancy"

8:30 AM Frank C. Miller, M.D.

"Obstetrical Events Leading to

Mentally Retarded Children"

9:30 AM Julian P. Parer, M.D.

"Update on Rh Disease"

10:30 AM Frank C. Miller, M.D.

"Management of Multiple Gestations"

11:30 AM Julian P. Parer, M.D.

"Four Ways to Decrease C/Section Rate"

Sunday, November 3, 1985

7:30 AM Lawrence Platt, M.D.

"New Methods of Fetal Assessment Including

Biophysical Profile & Doppler Blood Flow Measurements"

8:30 AM Frank C. Miller, M.D.

"Antepartum & Intrapartum Management of Post-dated Pregnancies"

9:30 AM Lawrence Platt, M.D.

"Use of Ultrasound in Labor & Delivery"

10:30 AM Jerry Wiley, J.D.

"Is Suing Obstetricians the

New National Pastime?"

11:30 AM Lawrence Platt, M.D.

"Prenatal Diagnosis Update"

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THE CHEMISTRY IS RIGHT

While some short-term studies1-3 have shown significant lipid changes, separate, carefully controlled long-term studies have shown no significant changes in total cholesterol, HDL cholesterol and triglycerides with NORDETTE at 12 months.*4-6

References:
1. Rossner S et al: Acta Obstet Gynecol Scand 59;255, 1980.
2. Nash AL et al: Med J Aust 2:277, 1979. 3. Larsson-Cohn U et al: Fertil Steril 35:172, 1981. 4. Briggs MH: J Reprod Med 28:92, 1983. 5. Åhrén T et al: Contraception 24:451, 1981.
6. Briggs MH, Briggs M: Acta Obstet Gynecol Scand Suppl 105:25, 1982.

Serious as well as minor adverse reactions have been reported following the use of all oral contraceptives

See important information on reverse page

ULTRA LOW DOSE

THE ULTRA LOW DOSE ORAL CONTRACEPTIVE WITH THE RIGHT PROFILE.

Available in 21- and 28-day dosage regimens.

es and Usage —NORDETTE* is indicated for the prevention y in women who elect to use oral contraceptives (OC's) as a

OC's should not be used in women with any of the wing conditions: 1. Thrombophlebitis or thromboembolic dis 2. A past history of deep-yein thrombophiebitis or thromboembolic disor-2. A past history of deep-vein thrombophilabilis or thromboembolic dispenses. 3. Cerebral-vascular or coronary-arrey disease. 4. Known or suspected carcinoma of the breast. 5. Known or suspected estrogen-dependent neoplasia. 6. Undiagnosed abnormal genital bleeding. 7. Known or suspected pregnancy (see Warning No. 5), 8. Benign or malignant liver tumor which developed during use of OC's or other estrogen-containing products.

Cigarette smeking increases the risk of serious cardiovascular aide effects from eral contraceptive use. This risk increases with age and with heavy smeking (15 or mere cigarettes per day) and its quite marked is women over 35 years of age. Women who use eral contraceptives should be strengly advised not to smeke. The use of oral contraceptives is associated with increased risk of several serious conditions, including thromboembolism, stroke, myocardial infarction, hepatic adenoma, galibladder disease, hypersension, Parchitioners neception and contraceptives behald be.

tension. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks.

Intromboembolic Disorders and Other Vascular Problems—An increased risk of thromboembolic and thrombotic disease associated with use of OC's is well established. Three principal studies in Great Britain and 3 in the U.S. have demonstrated increased risk of fatal and nonfatal venous thromboembolism and stroke, both hemorrhagic and thrombotic. These studies estimate that users of OC's are 4 to 11 times more likely venous thromboembolism and stroke, both hemorrhagic and thrombotic. These studies estimate that users of 0°C s are 4 to 11 times more likely than nonusers to develop these diseases without evident cause. CEREBROVASCULAR DISORDERS—In a collaborative American study of cerebrovascular disorders in women with and without predisposing causes, it was estimated that the risk of hemorrhagic stroke was 2.0 times greater in users than nonusers and the risk of thrombotic stroke was 4 to 9.5 times greater in users than in nonusers. MYOCARDIAL INFARCTION (MI)—An increased risk of MI associated with the use of O°C's has been reported, confirming a previously suspected association. These studies, conducted in the UK, found, as expected, that the greater the number of underlying risk factors for condary-artery disease (cigarette smoking, hypertension, hypertension, hypertension, hypertension, bestry, diabetes, history of pre-eclamptic toxemia) the higher the risk of developing MI, regardless of whether the patient was an OC user or not. OC's, however, were found to be a clear additional risk factor. In terms of relative risk, it has been estimated that OC users who do not smoke (smoking is considered a major predisposing condition to MI) are about twice as likely to have a fatal MI as nonusers who do not smoke. OC users who are also smokers have about a 5-fold increased risk of fatal MI compared to users who do not smoke, but about a 10- to 12-fold Lo users who are also smokers have about a 5-rold increased risk of tatal MI compared to users who do not smoke, but about a 10-to 12-rold increased risk compared to nonusers who do not smoke. Furthermore, amount of smoking is also an important factor. In determining importance of these relative risks, however, baseline rates for various age groups must be given serious consideration. Importance of other predisposing conditions mentioned above in determining relative and absolute risks has not as yet been quantified; quite likely the same synergistic action exists but perhaps to a lesser extent.
RISK OF DOSE—In an analysis

but perhaps to a lesser extent. RISK OF DOSE—In an analysis of data derived from several national adverse-reaction reporting systems. British investigators concluded that risk of thromboembolism, including coronary thrombosis, is directly related to dose of estrogen in OC's. Preparations containing 100 mcg or more of estrogen were associated with higher risk of thromboembolism than those containing 50-80 mcg. Their analysis did suggest, however that quantity of estrogen may not be the sole factor involved. This finding has been confirmed in the U.S.

ESTIMATE OF EXCESS MORTALTY FROM CIRCULATORY DISEASES—A street prospective study exartied out in the U.K.

ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASES—A large prospective study carried out in the UK estimated the mortality rate per 100,000 women per year from diseases of the circulatory system for users and nonusers of OC's according to age, smoking habits, and duration of use. Overall excess death rate annually from circulatory dis-eases for OC users was estimated to be 20 per 100,000 (ages 15-34— 5/100,000; ages 35-44—33/100,000; ages 45-49—140/100,000), risk being concentrated in older women, in those with long duration of use, and in cigarette smokers. It was not possible, however, to examine inter-relationships of age, smoking and duration of use, on the compact and in cigarette smokers. It was not possible, however, to examine inter-relationships of age, smoking, and duration of use, nor to compare effects of continuous vs. intermittent use. Although the study showed a 10-fold increase in death due to circulatory diseases in users for 5 or more years, all these deaths occurred in women 35 or older. Until larger numbers of women under 35 with continuous use for 5 or more years are available, it is not possible to assess magnitude of relative risk for this younger group. Available data from a variety of sources have been ana-lyzed to estimate risk of death associated with various methods of contra-ception. Estimates of risk of death for each method include combined risk of contraceptive method (e.g., thromboerbolis) and thrombotic disease in ception. Estimates of risk of death for each method include combined risk of contraceptive method (e.g., thromboembolic and thromboth disease in the case of OC's) plus risk attributable to pregnancy or abortion in event of method failure. This latter risk varies with effectiveness of method. The study concluded that mortatily associated with all methods of birth control is low and below that associated with childbirth, with the exception of OC's in women over 40 who smoke. Lowest mortality is associated with condom or diaphragm backed up by early abortion. Risk of thromboembolic and thrombothe disease associated with OC's increases with age after about 30 and, for MI, is further increased by hypertension, hypercholesterolemia, obesity, diabetes or history of pre-eclamptic toxemia, and especially cigarette smoking. Physician and patient should be alert to earliest manifestations of thromboembolic and thrombothe disorders (e.g., thrombophlebitis, outmonary embolism, cerebrovascular insuffiearliest manifestations of thromboembolic and thrombolic disorders (e.g., thrombophlebits, pulmonary embolism, cerebrovascular insuffi-ciency, coronary occlusion, retinal thrombosis, and mesenteric thrombo sisis). Should any of these occur or be suspected, the drug should be discontinued immediately, A 4- to 6-fold increased risk of postsurgery thromboembolic complications has been reported in OC users. If feasible, OC's should be discontinued at least 4 weeks before surgery of a type associated with increased risk of thromboembolism or pro-langed immobilization.

longed immobilization PERSISTENCE OF RISK OF VASCULAR DISORDERS—Findings from one PERSITENCE OF RISK OF VASCULAR DISORDERS—Findings from one study in Britain involving cerebrovascular disease and another in the U.S. concerning MI suggest an increased risk of these conditions in users of OC's persists after discontinuation of the OC's. In the British study, risk of cerebrovascular disease remained elevated in former OC users for at least 6 years after discontinuation. In the U.S. study, increased risk of MI persisted for at least 9 years in women 40 to 49 years old who had used OC's for 5 or more years. Findings in both studies require confirmation since they are inconsistent with other published information. 2. Ocular Lesions—There have been reports of neuro-ocular lesions such as optic neuritis or retinal thrombosis associated with use of OC's. Discontinue OC's if there is unexplained, sudden or gradual, partial or complete loss of vision; onset of protospis or dicining anailledema or

complete loss of vision; onset of proptosis or diplopia; papilledema; or retinal-vascular lesions, and institute appropriate diagnostic and therap tic measures

Carcinoma-Long-term continuous administration of either natural

or synthetic estrogen in certain animal species increases frequency of carcinoma of the areast, cervix, vagina, and liver Certain synthetic progestogers, nane currently contained in OC's, have been noted to increase incidence of mammary nodules, benign and malignant in dogs. In humans, 3 case-coeutrol studies have reported an increased risk of endometrial carcinoma associated with prolonged use of exogenous estrogen in postmenosausal women. One publication reported on the first 21 cases submitted by physicians to a registry of cases of adenocarcinoma of the endometrium in women under 40 on OC's. Of cases found in women withous-presisposing risk factors (e.g. irregular bleeding at the time-OC's were first given, polycystic ovaries), nearly all occurred in women who had used a sequential OC. These are no longer marketed. No women who had used a sequential OC. These are no longer marketed. No evidence has been reported suggesting increased risk of endometrial cancer in users of convenional combination or progestogen-only OC's. Several studies have 15und ho increase in breast cancer in women taking OC's or estrogens. One study, however, while also noting no overall increased risk of irread cancer in women on OC's, found an excess risk in subgroups of CC users with documented beingin breast disease. Reduced occurrence or beingin breast timors in users of OC's has been well documented. In summary, there is all present no confirmed evidence from human studies of increased risk of cancer associated with OC's. Close clinical surveillance of all women on OC's is nevertheless lessen

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Close clinical surveillance of all women on OC's is, nevertheless essential. In all cases or unclagnosed persistent or recurrent abnormal vaginal bleeding, appropriate larginostic measures should be taken to rule out malignancy. Women with a strong family history of breast cancer or with breast nodules, firercestic disease, or abnormal mammograms should be monitored with particular care if they elect to use OC's.

4. Hepatic Tumors—Benigh hepatic adenomas have been found to be associated with use of OC's. One study showed that OC's with high hormonal potency were associated with higher risk than lower potency OC's. Although beingn, respace adenomas may rupture and may cause death through intra-abdominal hemorrhage. This has been reported in short-term as well as long-term users. Two studies relate risk with duration of use of OC's, the risk being much greater after 4 or more years' use. While hepatic adenoma is rare, it should be considered in women presenting abdominaupair and tenderness, abdominal mass or shock. A tew cases of hepatocesular carcinoma have been reported in women on OC's. Relationship of these crugs to this type of malignancy is not known.

Relationship of these crugs to this type of malignancy is not known.

5. Use in or immediately Preceding Pregnancy. Birth Defects in Offspring, and Malignancy in-Fernale Offspring—Use of female sex hormones—both estrogenic and progestational agents—during early pregnancy may seriously damage the effspring. It has been shown that females exposed in utero to disethystitibestrol, a nonsteroidal estrogen, have increased risk of developing in later lies a form of vaginal or cervical cancer ordinarily extremely rare. This risk has been estimated to be of the order of 1 in 1,000 exposures or less. Although there is no evidence now that OC's further enhance risk offideveloping this type of malignancy, such patients should be monitored with particular care if they elect to use OC's. Furthermore. 30 to 99% of such exposed women have been found to have eighthelial changes after vagina and cervix. Although these changes are histologically benion. It is not known whether this condition is a precursor of vaginal malignancy, valid children so exposed may develop abnormalities of the urogenial tract. Although similar data are not available with or veginal maigraery, wate children so exposed may develop anormalities of the urogenial tract. Although similar data are not available with use of other estrogens, it cannot be presumed they would not induce similar changes. An interessed risk of congenital anomalies, including heart defects and limb defects, has been reported with use of sex hormones, including 6°Cs in pregnancy. One case-control study estimated a 4.7-fold increase in risk of imb-reduction defects in intants exposed in utero to sex hormones (0°Cs, hormonal withdrawal tests for pregnancy, or attempted treatmentation threatment abortion). Some exposures involved only a few daya. Data suggest that risk of limb-reduction defects in exposed fetuses-is symewhat less than 1 in 1,000 live births. In the past, female sex hermones have been used during pregnancy in an attempt to treat thi-eatewed or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well-controlled studies that progestogens are effective. There is some evidence that triploidy and possibly other types of polyploidy are increased among abortiuses from women who become pregnant soon after ceasing O°Cs. Embryos with these anomalies are virtually always aborted spentareously. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping O°Cs. ties of the urogenital tract. Although similar data are not available with always abortled spentameously. Whether there is an overall increase in spontaneous abortlon of pregnancies conceived soon after stopping OC's sunknown. It is recommended that for any patient who has missed 2 consecutive periods, pregnancy should be ruled out before continuing OC's. If the patienthmas not adhered to the prescribed schedule, the possibility of pregnancy should be considered at time of first missed period, and further use of OC's. Should be withheld until pregnancy has been ruled out. If pregnancy is confirmed, the patient should be apprised of the potential risks to the fetus, and advisability of continuation of the pregnancy should be discussed. It is also recommended that women who discontinue OC's with intent of becoming pregnant use an alternate form of contraception for a period of time before attempting to conceive. Many clinicians recommend 3-months, although no precise information is available on which to base this. The administration of progestogen-estrogen combinations to induce withdrawal biseding should not be used as a test of pregnancy.

-Studies report increased risk of surgically con ned gallbladder disease in users of OC's and estrogens. In one study, reased risk appeared after 2 years use and doubled after 4 or 5 years use. In one of the other studies, increased risk was apparent between 6

use. In one of the other studies, increased risk was apparent between 6 and 12 months' use.

7. Carbohydrate and Lipid Metabolic Effects—Decrease in glucose tolerance has been observed in a significant percentage of patients on OC's. For this reason, prefliabetic and diabetic patients should be carefully observed while on \(\theta\)C's. Increase in triglycerides and total phospholipids has been observed in patients on OC's, clinical significance of this finding remains to be defined.

8. Elevated Blood Fressiere—Increase in blood pressure has been reported in patients in CC's. In some women, hypertension may occur.

within a few months of Beginning OC's. In the 1st year of use, prevalence of women with hypertension is low in users and may be no higher than that of a comparable group of nonusers. Prevalence in users increases, however, with longer exposure, and in the 5th year of use is 2½ to 3 times the reported prevalence in the 1st year. Age is also strongly correlated with development of hypertension in OC users. Women who previously have had hypertension during pregnancy may be more likely to develop elevation of blood pressure on OC's. Hypertension that develops as a result of taking:DC's usually returns to normal after discontinuing the drug

-Onse: or exacerbation of migraine or development of head-

9. Meadache—Onse: or exacerbation of migraine or development of headache of a new pattern which is recurrent, persistent, or severe, requires discontinuation of CE's and evaluation of the cause.
10. Bleeding Irregularities—Breakthrough bleeding, spotting, and amenorrhea are frequent reasens for patients discontinuing OC's. In breakthrough bleeding, as in all cases of irregular vaginal bleeding, nontunctional causes should be binone in mind. In undiagnosed persistent or recurrent abnormal ielecting from the vagina, adequate diagnostic measures are indicated in rule out pregnancy or malignancy. If pathology has been excluded, timezin change to another OC may solve the problem.
Changing to an OC with a higher estrogen content, while potentially useful

in minimizing menstrual irregularity, should be done only if necessary, since this may increase risk of thromboembolic disease. Women with since this may increase risk of thromboembolic disease. Women with past history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may have a tendency to remain anovulator to become amenorrheic after discontinuing OC's. Women with these preexisting problems should be advised of this possibility and encourag to use other methods. Post-use anovulation, possibly prolonged, may also occur in women without previous irregularities.

11. Ectopic Pregnancy—Ectopic as well as intrauterine pregnancy may occur in contraceptive failures. ry vvulatory

occur in contraceptive failures.

12. Breast-feeding—OC's given in the postpartum period may interfere with lactation and decrease quantity and quality of breast milk. Furthermore, a small fraction of the hormones in OC's has been identified in the milk of mothers on OC's effects. If any, on the breast-jed child have not been determined. If feasible, defer OC's until infant has been weaned.

Precartiess—OENERAL—1. A complete medical and family history should be taken prior to initiation of OC's. Perteratment and periodic physical examinations should include special reference to blood pressure.

physical examinations should include special reference to blood pressure breasts, abdomen and pelvic organs, including Pap smear and relevant laboratory tests. As a general rule OC's should not be prescribed for longer than 1 year without another physical examination.

2. Under influence of estrogen-progestogen preparations, preexisting uterine leiomyomata may increase in size.

3. Patients with history of psychic depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. Patients becoming significantly depressed while on OC's should stop OC's and use an alternate method to try to determine whether the symptom is discontinued.

symptom is drug-related.

4. OC's may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients wit conditions which might be aggravated by fluid retention, such as convulsive discretes, migraine syndrome, asthma, or cardiac or renal

Insufficiency

5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence while on OC's. If jaundice develops, OC's

Stitution be discontinued.

6. Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution.

7. OC users may have disturbances in ormal tryptophan metabolism which may result in a relative pyridoxine deficiency. Clinical significance is

undetermined. 8. Serum folate levels may be depressed by OC's. Since the pregnant woman is predisposed to development of folate deficiency and incidence of folate deficiency increases with increasing gestation; it is possible that if a woman becomes pregnant shortly after stopping OC's, she may have a greater chance of developing folate deficiency and complications attributed to the deficiency. uted to this deficie

uted to this deficiency.

Information are the Patient—See Patient Package Labeling.

Laberatory Tests—1. The pathologist should be advised of OC therapy when relevant specimens are submitted.

2. Certain endocrine- and liver-function tests and blood components may be affected by estrogen-containing OC's:

a. Increased sulfobromophithalein retention.

b. Increased prothrombin and factors VII. VIII. IX. and X: decreased antithrombin. 3: uncreased no programphic production of the programphic programphi

b. Increased profitrombin and factors VII, VIII, IX, and X, decreased antithrombin 3; increased norepinephrine-induced platelet aggregability. c. Increased thyroid-binding globulin (186) leading to increased circulating total-thyroid hormone, as measured by protein-bound iodine (PBI). T4 by column, or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated T8G; free T4 concentration is unalitered. d. Decreased pregnanediol excretion. e. Reduced response to metyrapone test.
Drug Interactions.—Reduced efficacy and increased incidence of breakthrough bleeding have been associated with concomitant use of rifampin. A similar association has been suggested with backfurstes, phenyibuts.

unuqui olegoling nave been associated with concomitant use of rifampin A similar association has been suggested with barbiturates, phenylbuta-zone phenyton sodium, ampicillin and tetracycline Carcinopenesis, Michagenesis, Impairment of Fertility—See Warnings section #3.4, and 5 for information on carcinogenesis, mutagenesis, and impairment of fertility.

and imparment or tertuity.

Prepanary—Category X. See Contraindications, Warnings.

Nursing Methers—See Warnings. Because of the potential for adverse reactions in nursing infants from oral contraceptive tablets, a decision should be made whether to discontinue the drug, Taking into account the mportance of the drug to the mother.

Adverse Reactions—An increased risk of these serious adverse reactions

APPATHE RESCRIBES —An increased risk of these serious adverse react has been associated with use of OC's (see Warnings): hirrombophish pulmonary embolism, coronary thrombosis, cerebral thrombosis, cer bral hemorrhage, hypertension, galibladder disease, benign hepatoma congenital anomalies. There is evidence of an association between the following conditions and use of OC's although additional confirmatory studies are needed; mesenteric thrombosis, neuro-ocular lesions, e.o. retinal thrombosis and ootic neuritis

retinal thrombosis and optic neuritis.

The following adverse reactions have been reported in patients on OC's and are believed to be drug-related. Nausea and/or vomiting, usually the most common adverse reactions, occur in approximately 10 percent or less of patients during the first cycle. Other reactions, as a general rule, are seen much less frequently or only occasionally. Gastrointestinal symptoms (such as abdominal cramps and bloating), breakthrough bleeding, spotting, change in menstrual flow, dysmenorrhea, amenorrhea during and after treatment, temporary intertility after discontinuance of treatment; edema; chloasma or melasma which may persist; breast changes; tenderness, enlargement, and secretion; change in weight (increase or decrease); change in cervical erosion and cervical secretion; possible occusase; change in carvoar envision and cervical secretion; possible diminution in lactation when given immediately postpartum; cholestatic jaundice; migraine; increase in size of uterine leiomyomata; rash (allergic); mental depression; reduced tolerance to carbotydrates; vaginal candidiasis; change in corneal curvature (steepening), intolerance to con-

The following adverse reactions have been reported in users of OC's, and the association has been neither confirmed nor refuted; premenstrual-like syndrome cataracts, changes in libido, chorea, changes in appetite, cysti te syndrome, headache, nervousness, dizziness, hirsutism, loss of scalp hair, erythema multiforme, erythema nodosum, hemorrhagic erup

Stephysian by man annument of the stephysics of



each tablet contains 0.15 mg levonorgestrel and

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CLINICAL SECTION

Clinical Opinion

Dickinson's sign: Focal uterine softening in early pregnancy and its correlation with the placental site

Robert A. Munsick, M.D., Ph.D.

Indianapolis, Indiana

Focal uterine softening, characteristic of early pregnancy, is usually known as Piskacek's or von Braun–Fernwald's sign but should be attributed to Robert Latou Dickinson, who first described it in 1892. The presence or absence of Dickinson's sign was carefully noted in 1040 pregnant patients seeking abortions. The weeks of gestation, location of uterine softening, and correlation with placental site were also studied. This sign reliably showed the placental site, hence its unequivocal presence nearly proves the presence of intrauterine pregnancy and precludes the existence of tubal pregnancy. It was present in most pregnancies between 6 and 11 menstrual weeks, and its presence after 14 weeks should make menstrual dates suspect or signal the possibility of a septate or bicornuate uterus. (AM J OBSTET GYNECOL 1985;152:799-802.)

Key words: Uterine softening, Dickinson's sign

During the late nineteenth and early twentieth centuries, experience gained through the pelvic examination in early pregnancy caused many European and American gynecologists to publish their physical findings, some of which were given eponyms and were claimed to be diagnostic of early pregnancy. Diagnostic accuracy from the physical examination was mandatory, because tests for human chorionic gonadotropin did not exist then.

While studying how accurately one can estimate the duration of early pregnancy using only the pelvic examination, I thought that the presence or absence of one or more of these various signs of pregnancy might help to delimit the duration of gestation. Only one, focal uterine softening, or Dickinson's sign, 1.2 seemed incontrovertible and nearly free of subjective interpretation of its presence, absence, or extent. Therefore the prevalence and location of this sign were noted by week of gestation, and correlation of its location with the placental site was noted in a series of women under-

going abortion operations. Differences in its weekly prevalence and location were also analyzed according to parity. The results of this study were compared with those published near the turn of this century.

Material and methods

Between 1975 and 1983, 1040 pregnant women who requested abortion were studied. After they voided, they were carefully assessed by me, with use of customary methods of bimanual vaginal examination.3 Menstrual dates were unknown to me until after the examination was completed and the physical findings had been recorded. Uterine size and the location of any focally softened area were recorded, whether the softened area was small or occupied a majority of the uterine surface. To palpate such uterine irregularities of shape and consistency, the two fingers in the vagina were swept across the anterior and lateral uterine walls while the abdominally exploring fingers fixed the uterus and palpated its fundal and posterior surfaces. Sites of focal softening were recorded as anterior, posterior, left, right, or fundal. When the area was large, the predominant location was chosen. Ladin's sign,4 a fingertip-size area of softness in the uterine isthmus, is not the same as Dickinson's sign, and such cases were not considered to have focal uterine softening.

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Table I. Prevalence of focal uterine softening by gestational week

	All pr	egnant wor	nen ,	Nulli	parəvə we	men	. * Pa	irous wome	n	Ie	leal group	k
Week	Т	+	. %	Τ.		%.	T .	+	%	. T	+	%
6	6	.3	50	1)	0	4	3	75	4	2	50
7	20	9	45	8	1	50	12	5	42	12	6	50
8	31	16	52	12	วั	42	12	8	67	26	15	58
9	86	54	63	9	. 1	44	41	23	56	62	46	74
10	135	78	58 -	23	10	. 43	· 58	27	47	105	64	61
11	115	. 64	56	18	_ 7	39	59	31	53	98	57	58
12	119	42	35	29	3	28	58	21	36	103	41	40
13	108	37	34	27	· Э	33	53	18	· 34	100	35	35
14	97	29	30	26	∋	35	54	13	24	87	29	33
15	101	9	9	4 36	1	11	48	2	4	98	9	9
16	68	1	1	33	1	3	27	Ö	0	66	1	2
17	64	0	0	29)	0	29"	0	0	63	0	0
18	47	1	2	18	\supset	0	28	l	1	45	I	2
19	21	o o	0	11	Э.	0	10	0	ö	21	0	0
20	16	0	0 .	6	J	0	10	0	0	15	0	O.
21	4	0	0	1	J .	0	3	0	0	4	0	0
22	2	0	0		- .		2	. 0	0	2	0	0
Total	1040	343	33	287	ΕÌ	21	507	.152	30	911	306	34

T = Total observations, + = positive Dickinson's ser; and % = percent positive.

One or more laminaria tents were inserted into the cervix, and abortions were performed about 24 hours later by suction or evacuation. A clear plastic vacuum cannula was inserted through the cervix in such a direction that the opening was applied against the firm part of the uterus, 180 degrees from the location of softening. Suction was then begun, and the cannula was slowly rotated until placental tissue was aspirated. The location of the cannula opening at this time was recorded as the placental site. Immediately after the procedure was completed, fetal foot, leg, and arm beguns were measured with a vernier calipers to the recires 0.002 inch, even though such precision is neither ruly possible nor necessary.

All data were immediately recorded on a needesigned form and later were output with a NE PC-8001A microcomputer to a sequential file, by means of a Basic program designed to trap as many outputerors as possible. Input programs used to analyze data vere also written for this system.

Menstrual weeks of gestation were calculated by averaging the weeks computed from each extremity measurement, by means of previously reported fou to-order polynomial regression formulas. If no extremity was measurable, the week of gestation was determined from menstrual dates, when accurate; otherwise the estimate of uterine size from the vaginal bimanual examination was used.

Results

The prevalence of Dickinson's sign was first cetermined for all women at all menstrual weeks from 6 to 22. Results are shown in Table I. About one half had

focal uterine softening from 6 to 8 weeks and the peak prevalence was at 9 to 11 weeks, when more than half showed it. By 12 weeks most uteri had become generally soft; after 14 weeks few uteri showed any residual firmness, so the sign became unusual this late.

Focal softening was found in 31% of parous women but in only 21% of nulliparous women, a difference which was significant by the Fisher's exact test with two tails (p < 0.01). Because parity was not recorded in the early part of this study, the total of all nulliparous and parous women does not equal the total for all gravid women.

A final and possibly most accurate picture of the prevalence of Dickinson's sign is also shown in Table I. All cases of extreme obesity, leiomyomas, and uterine retroposition were excluded in order to elucidate its prevalence when the uterus was most easily palpated. The peak prevalence was seen at 9 weeks, when 74% of uteri demonstrated the sign. At 14 weeks, focal softening persisted in one third of the uteri, but at 15 weeks it had decreased to 9%.

The locations of focal softening are shown in Table II for nulliparous and parous gravid women. In nulliparous women the left side clearly predominated, the right side being next in occurrence and significantly more common than the anterior, posterior, or fundal positions. In parous women there was no such preponderance except that the fundal position was rarely seen. When dextro- and sinistropreponderance were compared for weeks 6 to 11 versus 12 and more, the left side appeared to predominate in both parous and nulliparous women in the later but not in the earlier weeks (Fisher's exact test with two tails, p = 0.01). The

^{*}Ideal group excludes all pregnant women with ut∈i⊐e retroposition, marked obesity, or uteri with leiomyomas.

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6

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Fundal

Totalt

	All pregna	nt women	Nulliparous women		Parous women		Ideal group*	
Location .	n	%	n	%	n	%	n	%
Left	127	37	29	48	41	27	120	39
Right	89	26	17	28	40	26	71	23
Anterior	62	18	6	10	33	22	56	18
Posterior	58	17	9	15	36	24	53	17

O

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Table II. Location of Dickinson's sign according to parity and ease of examination

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apparent differences in left-sided preponderances between nulliparous and parous women were not statistically significant.

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344

The placental site was located by the vacuum curette in 233 (68%) of the 343 uteri in which Dickinson's sign was present. The two sites were similarly located in 205 (88%), 90 degrees disparate in 26 (11%), and 180 degrees apart in two (1%) cases.

In the entire group of 1040 pregnant women eight were found to have septate or double uteri. Dickinson's sign was present in the following numbers: 9 weeks, 0 of 1; 12 weeks, 1 of 1; 13 weeks, 0 of 1; 14 weeks, 2 of 2; 15 weeks, 1 of 2; 16 weeks, 0 of 1. There were too few cases in this group to compare them statistically with all gravid women, but it seems highly likely that focal softening persists longer when the cavities are separated. For the four cases with focal softening, there was perfect agreement between the side of softness and the gravid side of the uterus.

Comment

In 1892 Robert Latou Dickinson briefly described the irregularities in shape and consistency which are so characteristic of early pregnancy.1 He provided more details from a larger series of patients in 1893,2 noting that there was "bellying" of the uterine wall, that "furrows" developed transversely or longitudinally on the anterior wall, and that areas of contrasting firmness and softness occurred even before 8 menstrual weeks of pregnancy. Although he focused particularly on the grooves and furrows, which probably represent the junction of soft with firm uterine wall, and on the firm area of the uterus, which he thought represented the placental implantation site, there can be no question from his text and many drawings that he did indeed first describe focal uterine softening. In his classic paper of 1908, McDonald⁶ stressed Dickinson's primacy in this regard. In 1899 von Braun-Fernwald⁷ and Piskacek⁸ both described such softened areas in early pregnant uteri.

My original purpose in studying the weekly preva-

lence of focal uterine softening was to determine whether its presence might help to delimit the number of weeks of gestation, that is, might the sign, when elicited, only be present at one or two gestational weeks? McDonald⁶ and Weissenberg⁹ had already published data that failed to support my hypothesis, but these antique papers were unknown to me when this study was undertaken.

McDonald⁶ found that there was focal uterine softening in a majority of pregnant uteri before 11 weeks (40 of 68) but that symmetrical softening occurred more frequently after 10 weeks (25 of 32). He found some softening in all pregnant uteri, even at 5 weeks. Overall, he noted that the uterus was symmetrically softened (probably meaning anterior, posterior, both or entirely softened) in 53 of 100 patients and that it was softened on the left in 21 and on the right in 26. He opined that softening indicated the placental site. Weissenberg⁶ found that the soft area was usually on the left or right and seldom symmetrical. He also found that the left side predominated over the right by a ratio of 73 to 35 in the 108 women he studied and that this left-sided preponderance was consistent from 5 to 13

Most of the data in my study confirm McDonald's and Weissenberg's findings. Focal softening was found as early as 6 weeks and was common from 7 to 11 weeks (see Table I). There was a significant left-sided preponderance of Dickinson's sign in nulliparous women but an essentially even distribution of the sign in parous women. Contrary to Weissenberg's findings, the left side was proportionally more often involved after 10 weeks than before for both nulliparous and parous women, but neither of these differences was statistically significant nor do they seem clinically important.

In three cases of incomplete abortion Dickinson performed digital curettage and found that the placental site correlated with the firm area of the uterus.² Both Piskacek⁸ and McDonald,⁶ held that the softened area correlated with the placental site. My findings may have been subject to bias, for I knew where the soft area was

^{*}Group includes all pregnant women without marked obesity, uterine retroposition, or uteri with leiomyomas.

[†]Total of all pregnant women exceed the sums of nulliparous and parous women for reasons listed in the text.

before introducing the vacuum curette. Nevertheless, beginning aspiration 180 degrees away from it and slowly turning the cannula should have revealed many more cases with immediate placental aspiration if the placental site correlated at all well with the area of uterine firmness. On the contrary, there was 88% agreement between the soft area and the placental location, with 11% being only 90 degrees and 1% being 180 degrees removed. Therefore, the evidence strongly favors the conclusion that the soft area reflects $\exists h \in pla$ cental implantation site. A frequent uterine firzling in cases of missed abortion is a patchwork of soft and firm areas in the uterine wall. This may explain DicLinson's erroneous conclusion regarding the location of The placental site; each of his three observations was in a case of incomplete abortion.

Why should the myometrium be selectively s-ft=ned over the placental site in early pregnancy? It seems likely that the softening results either from mycenetrial relaxation or from increased vascularity in this locale. Although Csapo's progesterone "block" theory to has been criticized on many counts, it is tempting to postulate that the softening results from progesterone. In three early pregnancies of 10 to 12, 12, and 14-weeks, Kumar and Barnes¹² found placental and antiplacental myometrial progesterone concentration ratios of 1.6, 1.3, and 1.6, respectively. It may also be relevant that it is precisely at 7 menstrual weeks of pregnamy that the placenta takes over major steroid biosynthes a from the corpus luteum13 and that Dickinson's sign appears in the majority of uteri. Might one extrapolate further and predict that when focal uterine softening is =learly present or the uterus is generally softened, lute comy would not lead to abortion?

The number of observed anomalous uteri vas too small to derive meaningful statistics, but it appears likely from the data presented here that when the placenta is located in one hemiuterus, the contralateral side fails to soften as early as usual. This may result from a higher than normal progesterone gradient between the retroplacental myometrium and the myometrium of the nonpregnant uterine horn. The presence of Dickinson's sign in the second trimester hould therefore alert to the increased possibility of a sprate or double uterus.

An important deduction from the above observat ons is that when Dickinson's sign is present a placeraa underlies it or conversely that there is not a singleton

pregnancy in an extrauterine location. Dickinson himself stated that this is not always the case. He noted that slight differences in consistency do occur in nonpregnant nulligravid and parous uteri.14 This is certainly true, but I have never felt pronounced focal softening when there was no placenta in the uterus, nor have I yet felt pronounced focal uterine softening when there was a tubal pregnancy, except when it was interstitial. Therefore the sign must be considered an excellent criterion of intrauterine or interstitial pregnancy.

Practical use can also be made of Dickinson's sign in abortion practice. By directing the opening of the vacuum curette toward the softened area, the placenta is usually rapidly aspirated, probably diminishing blood loss and often saving patients moments of unnecessary pain.

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Clinical Articles

The mortality risk associated with hysterectomy

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To study the risks of mortality associated with hysterectomy that are specific to age, race, surgical approach, and associated conditions, we used data collected by the Commission on Professional and Hospital Activities during 1979 and 1980. Four hundred seventy-seven deaths were recorded among 317,389 women having abdominal hysterectomies and 46 deaths among 119,972 women having vaginal hysterectomies. The mortality rates for hysterectomy, standardized for age and race, were higher for procedures associated with pregnancy or cancer than for procedures not associated with these conditions (29.2, 37.8, and 6.0 per 10,000 procedures, respectively). Hysterectomies associated with pregnancy or cancer constituted 8% of all hysterectomies performed. However, 61% of all deaths occurred in women with pregnancy- or cancer-related conditions. The mortality rate associated with hysterectomy increased with age and was twice as high among black women. (AM J OBSTET GYNECOL 1985;152:803-8.)

Key words: Mortality; hysterectomy, abdominal; hysterectomy, vaginal; risk

Hysterectomy is one of the most frequently performed operations on women of reproductive age in the United States.¹ Although nearly half a million women undergo the procedure each year,¹ few recent studies have examined the risk of mortality associated with hysterectomy. A few studies permit computation of mortality risks by factors that influence risk, such as age, race, and surgical approach. However, no study has examined mortality risks by associated medical conditions. For example, hysterectomies performed to treat malignancy and those performed concurrent with pregnancy termination are expected to be higher risk procedures and need to be analyzed separately.

Two studies of risks associated with hysterectomy reported mortality rates of 26.4 and 16.6 per 10,000.^{2.3} However, these were comparatively small studies conducted with data collected more than 10 years ago (Table I). In addition, the Commission on Professional and Hospital Activities (CPHA) published mortality rates of 17 and 8 per 10,000 women for abdominal and vaginal procedures, respectively, performed in Professional Activity Study (PAS) hospitals during 1972 and 1973.⁴ Although CPHA rates were specific to age and race, they were not detailed by other surgical risk factors.

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In the present study, we examined the risk of mortality specific to age, race, and surgical approach for nonradical hysterectomies associated with three categorizations of diagnoses: (1) diagnoses of cancer, (2) complications of pregnancy and delivery, and (3) all other diagnoses.

Methods

We obtained the data for our study from CPHA, which collected information from approximately 40% of patients discharged from short-stay hospitals in the United States in 1979 and 1980 (Commission on Professional and Hospital Activities, personal communication). From the PAS files, we selected the 441,762 records of all women discharged from the hospitals during 1979 and 1980 with procedure codes 68.3 (subtotal abdominal hysterectomy), 68.4 (total abdominal hysterectomy), and 68.5 (vaginal hysterectomy), according to the International Classification of Diseases, ninth revision, Clinical Modification (ICD-9-CM). We excluded 4015 duplicate records, 144 records that had hospital discharge status coded as unknown, 177 records that had both vaginal and abdominal hysterectomy codes, and 65 records that contained diagnostic and procedure codes that refer to men only.* A total of 437,361 women remained for analysis.

We categorized the conditions associated with hys-

*Male diagnostic codes: 185.0 to 187.9, 222.0 to 222.9, 233.4 to 233.6, 236.4 to 236.6, and 600.0 to 608.9, male procedure codes: 60.0 to 64.9.

Table I. Hysterectomy mortality rates in other ± dies

Authors	Descripte - 5 sample	No. of deaths	No. of women	Rate*
Cava ⁸	All nonradical _y=terectomies performed fr== 10/72 to 9/73 in a communate hospital of 400 beds	2	235	85.1
White et al. ⁷	Consecutive same es of 100 vaginal and II abdominal hysterectomics performed during 1958 to First in each of three setting private, clinic, and universit	4	600	66.7
Amirikia and Evans ²	All hysterecton = ⊃erformed at Hutzel Hosp = from 1965 to 1974	. 17	6,435	26.4
Leventhal and Lazarus ^e	First 300 vaging and 300 abdominal hearerctomies performed at McCarel Reese Hospital on remen 20 years of age and older a 1946	1	600	16.7
Ledger and Child ³	All hysterectonic in a sequential sample of e seventh discharge from CPHA-PAS hospitals dur. 1/70 through 6/70	20	12,026	16.6
CPHA Hospital Mortality ¹	All nonradical reterectomies reported by IHA-PAS hospi- tals in 1972 === 1973	737	506,250	14.6
Dicker et al. ¹¹	All nonemergency hysterectomies performed in the hospitals on women at 15 to 44 from 9/78 to 8/81	2	1,851	10.8
Pratt ¹²	Consecutive same of vaginal hysterectomes performed during 10 years twomen aged 35 to 60	1	952	10.5
Gray ¹⁰	Consecutive sande of abdominal hysterectomes performed by one surgen.	1	2,421	4.1

^{*}Crude mortality rate per 10,000 women having: Lesterectomy.

terectomy in three ways. We defined a hysterecarry as pregnancy-related if the discharge record con ained any of the ICD-9-CM diagnostic codes from t ₹0.0 to 676.9 or any of the ICD-9-CM procedure codes from 72.0 to 75.9. These diagnostic and procedur = codes correspond to complications of and treatment. ssociated with pregnancy, childbirth, and the puer rium. We defined a hysterectomy as cancer-related if the discharge record contained any of the ICD-9-C 4 diagnostic codes from 140.0 to 184.9 or from 188.0 \equiv Ξ 08.9; these two ranges of codes correspond to all amary and secondary invasive carcinomas. Finally, we defined a hysterectomy as "other" if the discharge read did not contain any ICD-9-CM diagnostic or pracdure codes that correspond to pregnancy, childbit, the puerperium, or invasive carcinoma. In situ caricomas of the cervix (ICD-9-CM diagnostic code 233 1 were included in the "other" group.

We defined deaths as those records in which ± 3 hospital discharge status was coded as dead. Because misclassification of deaths was reported in a study of mortality associated with tubal sterilization with the use of

the same data source,⁵ we attempted to assess misclassification of discharge status in our study. We reviewed all discharge diagnoses and procedures for women whose discharge status was recorded as dead and classified a death as "likely" if the record contained any of the ICD-9-CM diagnostic codes associated with lifethreatening complications such as cardiac arrest, shock, etc., or with procedures used to treat those complications, such as tracheostomy, dialysis, etc.* We classified a death as "questionable" if the discharge record did not have any of the diagnostic or procedure codes used to classify a death as "likely." Three hundred ninety-seven (76%) of the 523 deaths were categorized as "likely."

To simplify presentation, we did not analyze 7033 discharges from 1979 that were coded 68.2 (subtotal

*"Likely" death diagnostic codes: 276, 286, 415, 426 to 428, 430 to 438, 444, 466, 482, 486, 511.9, 514, 518, 960, 989, E87.0 to E87.6, 040.0, 348.1, 348.5, 349.0, 427.5, 518.4, 780.0, 780.2, 780.3, 785.4, 785.5, 995.2, 997.1 to 998.9; "likely" death procedure codes: 31.1, 34.04, 34.91, 37.7, 38.0, 38.7, 38.8, 39.3, 93.9, 96.0, 99.6, and 99.7.

Table II. Hysterectomy mortality rates* by surgical approach and associated conditions: CPHA, 1979 and 1980

				Surgical	approach	-			Total			
	,	Vagi	nal			Abdon	ninal					
	Pregnancy	Cancer	Other	Total	Pregnancy	Cancer	Other	Tetal	Pregnancy	Cancer	Other	Total
Discharges	464	1824	117684	119972	4971	26255	286163	317389	5435	28079	403847	437361
Maximum estimates							,					
No. of deaths	Ó	8	38	46	29	280	163	477	29	288	206	523
Crude mortality	0.0	43.9	3.2	3.8	58.3	106.6	5.9	15.0	53.4	102.6	5.1	12.0
rate ,												
Standardized	0.0	19.9	2.7	3.1	32.0	39.7	3.6	16.7	29.2	37.8	6.0	12.0
mortality rate†												
Minimum estimates												
No. of likely deaths	0	7 -	33	40	21	200	135	357	21	207	169	397
Crude mortality	0.0	38.4	2.8	3.3	42.2	76.2	4.8	11.2	38.6	73.7	4.2	9.0
rate			•									
Standardized mortality rate†	0.0	18.8	2.3	2.7	22.8	25.2	6.9	12.3	20.8	24.3	4.9	8.9

^{*}Per 10,000 procedures.

abdominal hysterectomy), 68.3 (total abdominal hysterectomy), and 68.4 (vaginal hysterectomy), according to the Hospital International Classification of Diseases Adaptation 2 (HICDA-2). These discharges were from hospitals that had not converted their CPHA reporting system from HICDA-2 to ICD-9-CM. Differences between ICD-9-CM and HICDA-2 coding schemes would complicate interpretation of results generated from combined data. These discharges represented less than 2% of the hysterectomy data available to us. Among these 7033 discharges, we identified 10 deaths among women who underwent abdominal hysterectomy and no deaths among women who underwent vaginal hysterectomy.

We used two methods to calculate mortality rates specific to associated conditions and surgical approach. First, we treated all reported deaths as representing maximum estimates of mortality associated with hysterectomy. Second, we treated only "likely" deaths as representing minimum estimates. The denominator for both sets of estimates was the total number of hysterectomies.

We also tabulated maximum mortality rates specific to age and race, separately for abdominal and vaginal approaches. The abdominal hysterectomy mortality rates were further subdivided by the three categories of associated conditions. Because the numbers involved were small, mortality rates specific to age and race were not estimated for the three categories of associated conditions for hysterectomies performed by the vaginal approach. Age and race were unknown for 0.1% and 6.7% of the discharges, respectively. By use of the direct method, all rates were standardized to the age and race distribution of all women in the CPHA files who underwent nonradical hysterectomy in 1979 and 1980.6

Results

The overall mortality rate associated with hysterectomy was 12 per 10,000 procedures (Table II). The mortality rates for both pregnancy- and cancer-related hysterectomies (29.2 and 37.8, respectively) were substantially greater than for hysterectomies performed for other reasons (6.0). Eight percent of hysterectomies were categorized as pregnancy- or cancer-related, and 61% of the deaths occurred in these subgroups.

There was an increased mortality risk for pregnancyand cancer-related hysterectomies with both surgical approaches. Among women who had abdominal hysterectomy, the mortality rates were 32.0, 39.7, and 8.6 for pregnancy-related, cancer-related, and other conditions, respectively. Among women who underwent vaginal hysterectomy, there were no pregnancy-related deaths. Among women who had cancer-related vaginal hysterectomy, the mortality rate was greater than for women who underwent hysterectomy for other conditions (19.9 and 2.7, respectively). In general, mortality rates for hysterectomies performed by the abdominal approach were higher than rates for hysterectomies performed by the vaginal approach.

Overall mortality rates increased with age, particularly after age 54 (Table III). This trend was also evident when the rates were subdivided by surgical approach. Further subdivision of abdominal hysterectomies according to associated conditions revealed an opposite trend among women who underwent pregnancy-related hysterectomy. The mortality rate for the

[†]Standardized by age and race of CPHA women uncergoing hysterectomy in 1979 and 1980; excludes 421 women whose age was unknown, 29,225 whose race was unknown, and 56 whose age and race were unknown.

Table III. Hysterectomy mortality rates* by age, surgical approach, and associated conditions: CPHA, 1979 and 1980

				Surgical approach		To	tal					
	Vaginal	(total)	Abdominal									
4	- Million Clark	-	Pregn	Pregnancy Cancer		Other		Total				
Age (yr)	Decihs	Rate	Deaths	Rate	Deaths	Rate	Deaths	Râte	Deaths	Rate	Deaths	Rate
<25	0	0.0	6	94.2	. 0	0.0	3	2.5	9	8.9	9	6.5
25-34	2	0.9	18	66.4	· 1	.7.1	16	2.3	35	4.7	37	3.5
35-44	4	0.5	5	34.5	6	26.8	32	2.8	43	3.8	47	3.1
45-54	4	2.7	0	0.0	18	41.4	33	4.6	51	6.5	55	5.8
55-64	3	1.9	0	0.0	63	80.4	28	19.0	91	41.3	.94	29.4
65-74	13	18.3	. 0	0.0	91	138.4	32	46.4	123	93.0	136	63.5
≥75	20	56.8	Ó	0:0	. 100	349.5	24	121.2	124	255.8	144	173.3

^{*}Per 10,000 procedures; standardized by race of CPHA women undergoing hysterectomy in 1979 and 1980; excludes 477 women whose age was unknown.

Table IV. Hysterectomy mortality rates* by race, surgical approach, and associated conditions: CPHA, 1979 and 1980

	Surgical approach										Total	
	Vaginal	(total)			Abdon	inal			Tot	al		
,			Pregn	ancy .	Can	cer	Oth	er				
Race	Deaths	Rate	Deaths	Rate	Deaths	Rate	Deaths	Rate	Deaths	Ratė	Deaths	Rate
White Black Other	37 5 0	2.8 6.6 0.0	19 8 2	28.9 51.7 65.5	228 34 1	36.7 69.2 5.8	110 47 0	7.9 15.7 0.0	357 89 . 3	16.0 24.5 6.3	394 94 3	11.0 21.3 4.4

^{*}Per 10,000 procedures; standardized by age of CPHA women undergoing hysterectomy in 1979 and 1980; excludes 29,282 women whose race was unknown.

latter was 94.2 per 10,000 among women less than 25 years of age. The rates decreased by approximately two thirds to 34.5 per 10,000 among women 35 to 44 years of age. The increased mortality rate for women who underwent pregnancy-related hysterectomy and who were less than 25 years of age accounted for the elevated total abdominal and overall rates for women less than 25 years of age.

The risk of death associated with hysterectomy was greater for black women than for white women (Table IV). The mortality rate for black women was nearly twice as high, irrespective of associated conditions or surgical approach.

When only the deaths that we classified as "likely" were used to estimate the risk of mortality associated with hysterectomy, the overall rate decreased to 8.9, with rates for pregnancy-related, cancer-related, and all others of 20.8, 24.3, and 4.9, respectively (Table II). We consider these rates to be minimum estimates of the risk of death associated with hysterectomy and note that they are not substantially different from the rates based on all reported deaths.

Comment

In our study, 92% of all hysterectomies were related to conditions other than pregnancy or cancer. Therefore, the mortality rate based on conditions other than pregnancy or cancer (6.0 per 10,000 women) seems more representative of the general risk of death associated with hysterectomy than the overall rate, which is twice as high.

The overall rate of mortality associated with hysterectomy in our study (12.0 per 10,000) is lower than reported by six studies^{2-1,7-9} and higher than reported in three studies¹⁰⁻¹² (Table I). Four studies enrolled fewer than 1000 women ^{7-9,12}; only the 1972-1978 CPHA-PAS series had sufficient numbers of deaths for a reasonable estimate of mortality rates specific to age, race, and surgical approach.⁴ However, unlike our study, the 1972-1973 CPHA rates were not subdivided by the presence of associated conditions.

The higher mortality rate seen for women less than 25 years old compared to women older than 25 who underwent pregnancy-related abdominal hysterectomy is based on six deaths in the former subgroup and could

be due to chance. Furthermore, we have no knowledge about whether these hysterectomies were planned preoperatively or performed as emergency, life-saving measures. The possibility exists that emergency hysterectomies were performed more often on younger women. The increased risk in women younger than 25 with pregnancy-related abdominal hysterectomy accounts for the increased risk seen in this same age group for all abdominal hysterectomies and for all hysterectomies.

The increased risk of death associated with hysterectomy among black women compared to white women has been reported previously.⁵ Furthermore, the rates are consistent with the reported increased risk of death among black women for pregnancy-related events.^{13, 14}

In our study, the risk of mortality for women undergoing an abdominal hysterectomy is greater than for women undergoing a vaginal hysterectomy. This finding is consistent with the risk of morbidity in one study11 and inconsistent with five studies.2, 3, 7-9 The differences between the mortality risk among women who underwent abdominal hysterectomy and that among women who underwent vaginal hysterectomy are difficult to interpret because in most studies, including ours, they may be attributable to selection bias. It is possible that women undergoing an abdominal hysterectomy in our study were, in the aggregate, at higher surgical risk than women undergoing a vaginal hysterectomy. For example, women with known gynecologic surgical risk factors, such as large pelvic masses, endometriosis, and pelvic inflammatory disease, are less likely to be considered candidates for the vaginal approach. Because we do not have preoperative clinical information available, we are unable to determine whether the observed differences are due to different surgical approaches or to differences in surgical risk factors for patients undergoing abdominal and vaginal procedures.

We believe that our results are representative of women undergoing hysterectomy in the United States for two reasons. First, they are based on large numbers of hysterectomies, approximately 40% of the hysterectomies performed during this time period in the United States. Second, our data on the number of hysterectomies performed in the United States compare favorably to data collected by the National Center for Health Statistics as part of the ongoing National Hospital Discharge Survey (NHDS) (ICD-9-CM codes 68.3, 68.4, 68.5), a probability sample of patients discharged from short-stay hospitals, exclusive of military and Veterans Administration hospitals located in the 50 states and the District of Columbia.15 CPHA estimates that 688,000 and 694,000 hysterectomies were performed. on women of all ages in the United States in 1979 and

1980 (Commission on Professional and Hospital Activities, personal communication); these compare to NHDS estimates of 635,000 and 647,000, respectively.¹⁵

Misclassification of deaths does not seem a probable explanation for our results. The minimum mortality rates for hysterectomy that are based only on death discharge records with life-threatening diagnoses and procedures are not substantially different than the rates based on all reported deaths. In an attempt to refine further both the minimum and maximum estimates, we examined length of hospitalization by surgical approach to see if the "likely" deaths were associated with different lengths of stay compared to those for the "questionable" deaths. The distribution of discharge records for unusual lengths of stay less than 6 days or greater than 3 weeks were similar for "likely" and "questionable" deaths. The absence of major differences in length of stay between "likely" and "questionable" deaths supports the credibility of the maximum estimates and lessens the likelihood that the "questionable" deaths have been misclassified.

Our analysis may underestimate the risk of mortality associated with hysterectomy. We have no information about possible rehospitalization and subsequent death as a result of complications stemming from the hysterectomies reported in our study.

In conclusion, our study of the risk of death associated with hysterectomy underscores the importance of analyzing pregnancy- and cancer-related conditions separately from all others. Without this refinement we would not have recognized that approximately 61% of all deaths associated with hysterectomy occurred in women with pregnancy- or cancer-related conditions, although such hysterectomies account for only 8% of hysterectomies performed. Using published data from a national probability sample¹⁵ and rates of mortality from our study, we estimate that approximately 770 (<1% of all women having a hysterectomy) women die annually in the United States as a result of having a hysterectomy. However, for the majority of hysterectomies performed for conditions other than pregnancy or cancer, we estimate that about 300 (39% of all deaths associated with hysterectomy) deaths occur each year. These mortality estimates allow women and their physicians to consider more specifically the risks and benefits of having a hysterectomy.

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Moxalactam versus clindamycin plus tobramycin in the treatment of obstetric and gynecologic infections

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The clinical efficacy of moxalactam versus clindamycin/tobramycin was evaluated in a comparative, randomized, prospective study. Sixty patients were treated: 30 with moxalactam and 30 with clindamycin/ tobramycin. There were 15 cases of tuboovarian abscess, 35 cases of severe pelvic inflammatory disease with peritonitis, eight cases of endomyometritis, and one wound abscess. Aerobic and anaerobic cultures from the sites of infection yielded 441 microorganisms from 53 patients; an average of 8.3 bacteria per infection (4.5 anaerobes and 3.8 aerobes). The infections tended to be mixed aerobic-anaerobic with anaerobes isolated in 90% of cases. The most frequently isclated possible pathogens were Bacteroides sp. (37), Bacteroides bivius (23), Bacteroides asaccharolyticus (12), Peptococcus asaccharolyticus (29), Peptostreptococcus anaerobius (19), unidentified anaerobic gram-positive cocci (18), Escherichia coli (17), nonhemolytic streptococci (16), Neisseria gonorrhoeae (13), and Gardnerella vaginalis (38). Clinical cure was noted in 29 of 30 moxalactam-treated and 29 of 30 clindamycin/tobramycin-treated patients. Moxalactam was effective in five of six cases of tuboovarian abscess, all 22 cases of pelvic inflammatory disease with peritonitis, the one case of endomyometritis and the one wound abscess. Clindamycin/ tobramyoin was effective in eight of nine cases of tuboovarian abscess, all 14 cases of pelvic inflammatory disease with peritonitis, and all seven cases of endomyometritis. No adverse hematologic, renal, or hepatic effects were noted with either regimen. (AM J OBSTET GYNECOL 1985;152:808-17.)

Key words: Pelvic infections, anaerobic-aerobic infection, moxalactam, clindamycintobramycin

The polymicrobial nature of most female genital tractinfections has led investigators to recommend antibiotic

treatment regimens that are effective against a wide variety of bacteria. These recommendations have often included an aminoglycoside in combination with penicillin, clindamycin, metronidazole, or even triple antibiotics. With the development of cephalosporins and penicillins with an expanded spectrum of activity against aerobic and anaerobic organisms, interest in the use of a single antibiotic rather than a combination of antibiotics to treat polymicrobial pelvic infections has

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More recently, moxalactam, a third-generation β -lactam antimicrobial agent with a broad spectrum of in vitro activity, has also been shown to be an effective single agent for the treatment of postpartum endometritis and pelvic inflammatory disease. ⁴⁻⁶ The current study was undertaken to compare the clinical efficacy and safety of moxalactam with that of a standard combination of tobramycin and clindamycin in the treatment of moderate to severe obstetric and gynecologic infections, with particular emphasis on gynecologic infections.

Material and methods -

From October, 1981, to April, 1983, 60 patients admitted to the obstetrics and gynecology service at San Francisco General Hospital were enrolled in the study. All patients signed an informed consent approved by the University of California (San Francisco) Committee on Human Research. According to a computergenerated randomized schedule, 30 patients received moxalactam at a dosage of 2 gm intravenously every 8 hours and 30 received 600 mg of clindamycin intravenously every 6 hours and 1.5 mg/kg of tobramycin every 8 hours.

Table I shows the demographic and clinical characteristics of the patients. Infections included 22 cases of acute salpingitis with peritonitis, six cases of tuboovarian abscess, one case of postabortal endometritis, and one case of wound abscess in the moxalactam group and 14 cases of acute salpingitis with peritonitis, nine cases of tuboovarian abscess, and seven cases of postpartum (two) or postabortal (five) endomyometritis in the clindamycin/tobramycin group. The criterion for diagnosis of a tuboovarian abscess was presence of a tender adnexal mass, which was confirmed by real-time ultrasound as a complex (cystic and solid) mass consistent with an abscess. In two instances diagnosis was confirmed at laparotomy. Three patients (two treated with moxalactam and one with clindamycin/tobramycin) had concomitant urinary tract infections.

Blood cultures, cervical cultures for the isolation of Neisseria gonorrhoeae, urine cultures, and/or cultures (in many cases including cultures for chlamydia) of the infection site were taken before the administration of the antibiotics. Cultures of the infection sites included 45 endometrial washes, three endometrial brushes, four culdocenteses, two peritoneal fluid samples, one cervical culture, and one wound culture. In addition, posttherapy endometrial cultures were obtained from 43 patients. All cultures from the infection sites and posttreatment endometrial cultures were transported to the laboratory in oxygen-free transport tubes and inoculated to media for the isolation of aerobic and

Table I. Characterization of study patients with genital tract infections

	Treatme	nt group
Parameter	Moxalactam	Clindamycin/ tobramycin
No. of patients	30	30
Age (yr)		
Range	17-39	17- 4 7
Mean ± SD	24.2 ± 5.1	24.7 ± 7.2
Race		
White	13	12
Black	13	11
Hispanic	4	5 2
Other	0	2 '
Gravidity		
Range -	. 0-7	0-11
Mean ± SD	1.9 ± 1.7	2.1 ± 2.3
Parity		
Range	0-4	0-9
Mean ± SD	1.2 ± 1.2	1.2 ± 1.9
Temperature (°C)		
Range	36.5 ± 40.1	37.0-40.0
Mean ± SD	37.7 ± 0.9	38.3 ± 0.9
White blood cell count ($\times 1000$)		
Range	4.7-18.0	6.0 - 23.5
Mean ± SD	-10.5 ± 4.0	12.1 ± 4.7
Duration of therapy (days)		
Range	2-11	2-12
Mean ± SD	5.9 ± 2.0	5.9 ± 2.6

anaerobic bacteria. Cultures for chlamydia from the same sites were transported to the laboratory in modified Eagle's medium broth. Cultures of the cervix for *N. gonorrhoeae* were inoculated directly to modified Thayer-Martin agar plates and transported to the laboratory in a candle jar. Blood for culture was injected at the bedside into bottles containing a supplemented trypticase soy broth.

Specimens obtained from the infection sites were inoculated into the following media for aerobic (carbon dioxide) incubation at 35° C: trypticase soy agar with 5% sheep blood, chocolate agar, modified Thayer-Martin agar, V agar. CNA agar, and MacConkey agar. Brucella agar with 5% sheep blood, vitamin K, and hemin and chopped meat carbohydrate broth incubated at 35° C in either a GasPak jar (BBL, Cockeysville, Maryland) or an anaerobic chamber (Coy Manufacturing, Ann Arbor, Michigan) were used for isolation of anaerobic bacteria. Anaerobically incubated CNA agar and kanamycin-vancomycin laked blood agar were added to the initial setup for about the final third of the study cultures.

Aerobically (carbon dioxide) incubated plates were examined two to three times over a 3- to 4-day period. Organisms were identified according to published procedures. N. gonorrhoeae was identified as an oxidase-positive, gram-negative diplococcus that produced acid from glucose but not from maltose, sucrose, or ONPG in the Minitek system (BBL, Cockeysville, Maryland). Anaerobically incubated plates were first examined af-

Table II. Comparison of clinical response in patients with pelvic infections treated with moxalactam or a clindamycin/tobramycin regimen

	Treatment group						
Infection	Moxalactam	Clindamycin/ tobramycin					
Acute salpingitis with peritonitis	22/22*	14/14*					
Tuboovarian abscess	5/6	8/9					
Endomyometritis	1/1	7/7					
Wound abscess	1/1	0					
Total	29/30	29/30					

^{*}Successful treatment per total infections.

ter 48 hours' incubation and several times thereafter for 7 to 10 days. Anaerobic bacteria were identified through the use of gas-liquid chromatography and prereduced anaerobically sterilized media.⁸

The chopped meat carbohydrate broth was subcultured after 3 to 5 days to media for both aerobic and anaerobic incubation. All organisms were identified as they were from the original cultures.

Blood cultures were examined for 7 days before being reported as having no growth. Organisms isolated from blood cultures were identified as described above.

Chlamydia trachomatis was isolated and identified through the use of cycloheximide-treated McCoy cells. The McCoy cells were inoculated with the clinical specimen by centrifugation, the culture medium was replaced, and the cells were cultured for 65 hours. The cells were then examined for inclusions demonstrated by iodine staining and immunofluorescence.

The minimal inhibitory concentrations of moxalactam, clindamycin, and tobramycin were determined by agar dilution methods. For anaerobic bacteria either Wilkins-Chalgren agar or Brucella agar supplemented with sheep blood and vitamin K was used as the base in which to incorporate the antibodies. For organisms that grew on both media, no differences in minimal inhibitory concentrations were noted.

Other laboratory determinations included complete blood count to monitor hematologic status and SMA-6 and SMA-12 to monitor renal and liver function status.

Results

Of the 60 women enrolled in the study, 29 of 30 (97%) who received moxalactam and 29 of 30 (97%) who received clindamycin/tobramycin were considered to be successfully treated (Table II). Both patients with treatment failure had been admitted with tuboovarian abscesses that were >8 cm in diameter and had had an

intrauterine contraceptive device in place for more than 8 years. Cultures obtained from the abscess wall in the patient with moxalactam treatment failure grew few nonhemolytic streptococci, not group B or D, and rare Fusobacterium necrophorum. The minimal inhibitory concentrations of moxalactam against the Fusobacterium was 0.5 µg/ml. An endometrial culture obtained from the patient with tobramycin/clindamycin treatment failure revealed occasional group D streptococci (not enterococci), occasional nonhemolytic streptococci, not group B or D, few Fusobacterium nucleatum, few Bacteroides sp. No. 1, and occasional Peptococcus asaccharolyticus and from the broth subculture only Bacteroides distasonis, Bacteroides asaccharolyticus, Bacteroides sp. No. 2, and Peptococcus magnus. The minimal inhibitory concentrations of clindamycin determined against the anaerobic bacteria F. nucleatum, both Peptococcus sp., Bacteroides sp. No. 1, and B. distasonis indicated that these organisms were susceptible to clindamycin. There was no aerobic or anaerobic growth from a peritoneal fluid culture taken several days later. Both of these patients improved following drainage of the abscesses accomplished through exploratory laparotomy in conjunction with total abdominal hysterectomy and bilateral salpingo-oophorectomy.

The treatment regimens were discontinued in three additional patients because of possible toxicity or allergic reactions. A pruritic, maculopapular rash was noted in a moxalactam-treated patient on the sixth day of therapy. The rash resolved after discontinuation of the moxalactam. A clindamycin/tobramycin-treated patient developed diarrhea on the fourth day of therapy and the antibiotics were discontinued 1 day earlier than planned. Both of these patients were afebrile at the time that the study antibiotics were discontinued and neither received any other antibiotics. Another clindamycin/tobramycin-treated patient was noted to have an increase in the serum creatinine level from 0.9 mg/dl on admission to 1.5 mg/dl on the fourth day of treatment. The patient was switched from clindamycin/ tobramycin to cefoxitin. The patient had received a 2 mg/kg loading dose of tobramycin followed by 1.5 mg/ kg every 8 hours. Unfortunately tobramycin levels were not obtained before the change in therapy. Prior to switching of the antibiotic regimens on day 5 of treatment, this patient had been afebrile for 48 hours and was considered to have a clinical cure; cefoxitin therapy was provided only for an additional 24 hours.

Three patients, all of whom were diagnosed as having postabortal endometritis and all of whom received clindamycin/tobramycin, also underwent dilatation and curettage because of uterine bleeding and concern over retained products of conception.

There were no other complications, side effects, or signs of toxicity related to the antibiotic treatment reg-

Table IIIA. Aerobic microorganisms isolated from pretreatment cultures of 55 infected women

		No. of isəla	tes	
Aerobic organism	Moxalactam group	Clindam scin/ tobramysin group	Total	From primary plates
Gram/positive cocci		•		
Group B streptococci	0	5	5	5
Group G streptococci	I	0	1	0
Enterococci	5	4	9	4
Group D streptococci, not enter- ococci	I	7	8	5
α-Hemolytic streptococci	. 5	ϵ	11	4
Nonhemolytic streptococci	7	č č €	16	9
Coagulase-negative staphylococci	9	Ę	18	14
Gram-negative cocci				
Neisseria gonorrhoeae	7	€	13	13
Gram-positive rods				
Lactobacilli	13	18	31	22
Diphtheroids	10	õ	19	18
Gram-negative rods				
Escherichia coli	7	10	17	13
Proteus mirabilis	1	()	1	1
Klebsiella pneumoniae	0 .	-	1	0
Acinetobacter calcoaceticus	0	- -	1	1
Alcaligenes sp.	1	0	1	1
Hemophilus influenzae	1	0	1	1
Gardnerella vaginalis	18	20	38	33
Unidentified coryneform organisms	6	3	9	7
Yeast				
Candida albicans	0	1	1	1
Total	92	10∋	201	152

imens during this study. Clinical signs of coagulopathy or platelet dysfunction were not seen in any study patients; however, laboratory evaluation of coagulation status was not included in the study protocol.

Fifty-six women had endometrial (48), cervical (one), wound (one), culdocentesis (four), or peritoneal (two) cultures taken prior to treatment. Three of these cultures showed no aerobic or anaerobic growth. From the other 53 cultures, 201 aerobic and 240 anaerobic organisms were cultured from both primary plates and broth subcultures (Tables IIIA and IIIB); this is an average of 8.3 different organisms per patient (4.5 anaerobes and 3.8 aerobes). If only primary plate cultures are taken into account, 152 aerobic and 133 anaerobic organisms were isolated from 48 cultures for an average of six organisms per patient (2.8 anaerobes and 3.1 aerobes).

N. gonorrhoeae was isolated from both endocervical and endometrial cultures in eight women. In an additional four women, N. gonorrhoeae was isolated only from endocervical cultures. In one instance, only the endometrial culture was positive for the organism.

The single most common isolate was Gardnerella vaginalis, isolated 33 times from primary cultures. While most of the cultures from which it was isolated were endometrial, it was isolated from two culdocentesis cultures along with other organisms. From 47% of the

cultures it was isolated in at least a moderate (2+) amount (colonies beyond the initial inoculum site); and from 66% it was present in greater amounts relative to the amounts of other aerobic organisms. However, the relative amounts of anaerobes were similar to or greater than the amount of Gardnerella in 68% of the cultures. In four cf five cases in which Gardnerella was isolated only from broth subculture, no organisms (aerobic or anaerobic) had been isolated from the primary culture plates although other organisms (usually anaerobic) were also isolated from the broth subculture.

Escherichia coli. N. gonorrhoeae, and group B streptococci were the most common of the aerobic isolates most likely to be pathogens. Haemophilus influenzae was isolated once as the only organism (other than lactobacilli and diphtheroids from the broth only) from an endometrial culture.

The most common anaerobic isolates were the grampositive cocci (99), especially peptococci, and Bacteroides species (90) including Bacteroides bivius (23), the Bacteroides melaninogenicus group (15), and a variety of Bacteroides species not further identified (37).

The minimal inhibitory concentrations of the study antibiotics were determined on the organisms most likely to be pathogens (Table IV). Moxalactam was more active than tobramycin against all of the aerobic bacteria except enterococci. However, moxalactam was

Table IIIB. Anaerobic microorganisms isolated from pretreatment cultures of 56 infected women

	No. of isolates						
Anaerobic organism	Moxalactam group	Clindamycin/ tobramycin group	Total	From primary plates			
Gram-positive cocci							
Peptococcus asaccharolyticus	11	18	29	16			
Peptococcus prevotii	2	4	6	3			
Peptococcus magnus	4	6	10	4			
Gaffkya anaerobia	5	5	10	8			
Peptostreptococcus anaerobius	8	11	19	10			
Anaerobic Streptococcus sp.	4	3	7	4			
Unidentified anaerobic gram-positive cocci	5	13	18	13			
Gram-negative cocci	*	-	c	0			
Veillonella parvula	I	5	6	2			
Acidaminococcus fermentans	2	1	3	I			
Megasphaera elsdenii Unidentified anaerobic gram-negative	1 2	0 0	1 2	0 1			
cocci		1					
Gram-positive rods							
Lactobacillus sp.	3	3	6	1			
Bifidobacterium sp.	5	1	6	2			
Actinomyces sp.	0	2	2	2			
Propionibacterium Sp.	1	0	1	1			
Unidentified gram-positive nonsporing rods	1	2	3	. 3			
Gram-negative rods							
Bacteroides bivius	. 11	12	23	17			
Bacteroides disiens	2	3	5	2			
Bacteroides fragilis group	$\tilde{1}$	3	4	2			
Bacteroides splanchnicus	Ô	Ī	î	ī			
Bacteroides asaccharolyticus	4	8	12	4			
Bacteroides melaninogenicus	î	9	3	2			
Bacteroides ureolyticus	· 3	2 2	5	1			
Bacteroides sp.	16	21	3 7	19			
Fusobacterium sp.	8	4	12	9			
Unidentified anaerobic gram-negative rods	4	5	. 9	5			
Total	105	135	240	133			

generally less active than clindamycin against most of the anaerobic bacteria tested. One isolate of *Peptostreptococcus anaerobius* from a pretreatment culture of a woman who was treated with moxalactam was resistant to moxalactam (minimal inhibitory concentrations >32 μ g/ml). One isolate of *P. anaerobius* and two isolates of *Bacteroides* from pretreatment cultures were only moderately sensitive (minimal inhibitory concentrations = 32 μ g/ml). However, these women recovered without other antibiotic treatment.

Posttreatment endometrial cultures were taken from 43 women. Of these, 11 (26%) showed no growth. Fiftynine aerobic and 29 anaerobic organisms were isolated from the remaining 32 cultures (Tables VA and VB), an average of 2.75 organisms per patient. N. gonorrhoeae was never isolated following either antibiotic treatment regimen. Yeast were isolated more frequently following moxalactam treatment, but enteric gram-negative rods were more common following clindamycin/tobramycin treatment, although these organisms were all susceptible in vitro to tobramycin (Table VI). Enterococci were isolated with approximately equal frequency from each

treatment group. In general, the women from whom enterococci were isolated after treatment were not the same women from whom enterococci were isolated prior to treatment. All women with these organisms recovered without incident.

All four posttreatment isolates of anaerobic grampositive cocci from women who were treated with clindamycin/tobramycin were resistant to clindamycin (Table VI). From women treated with moxalactam, one of four anaerobic gram-positive cocci isolates that were tested was resistant to moxalactam. The majority of posttreatment *Bacteroides* isolates were from women treated with moxalactam. Both *Bacteroides* fragilis group isolates and one unspeciated *Bacteroides* from women who received moxalactam were resistant to moxalactam. No clindamycin resistance among the anaerobic gram-negative rods was seen.

Pretreatment chlamydia cultures were obtained from 38 patients. Chlamydiae were isolated from one of 20 moxalactam-treated patients and one of 18 clindamycin/tobramycin-treated patients. The clindamycin-tobramycin-treated patient had a negative posttreatment

Table IV. Minimal inhibitory concentrations for bacteria isolated from pretreatment cultures

	Moxalactam			Clindamycin			Tobramycin*		
Organism	Range	MIC ₅₀	MIC ₉₀	Range	MIC ₅₀	MIC ₉₀	Range	MIC ₅₀	MIC ₉₀
Group B streptococci (n = 5†)	4-8	4	8		Not done		16-32	32	32
Enterococci (n = 8)	64->128	>128	>128		Not done		4-64	4	8
Neisseria gonorrhoeae (n = 13)	≤0.03-0.06	≤0.03	0.0€	4-8	8	8	4-16	8	16
Escherichia coli (n = 17)	≤0.03-0.125	0.06	0.125		Not done		0.5-1.0	0.5	1.0
Peptococcus asaccharo- lyticus (n = 28)	≤0.03-0.5	0.06	0.25	≤0.03-2.0	0.125	0.5			
Peptococcus prevotii (n = 6)	≤0.03-4	0.125	4	≤0.03-0.06	≤0.03	0.06			
Peptococcus magnus (n = 10)	0.125-4	0.5	4	≤0.06->64	0.5	2			
Gaffkya anaerobia $(n = 10)$	≤0.03-1.0	0.25	1.0	≤0.03-1.0	0.125	0.25			
Peptostreptococcus an- aerobius (n = 19)	0.25-64	2	32	≤0.03-0.25	≤0.03	0.25			
Veillonella parvula (n = 4)	≤0.06-16	1	16		≤0.06	≤0.06			
Bacteroides bivius (n = 23)	0.125-32	4	16		≤0.03	≤0.03			
Bacteroides disiens $(n = 5)$	0.125-4	2	4		≤0.03	≤0.03			
Bacteroides fragilis group $(n = 4)$	0.5-32	0.5	32	0.125-4	1	4			
Bacteroides asaccharo- lyticus (n = 5)	≤0.03-8	0.125	8		≤0.03	≤0.03			
Bacteroides melanino- genicus group (n = 3)	≤0.03-2	2	2		≤0.03	≤0.03			
Bacteroides sp. $(n = 15)$	0.125-32	4	16	≤0.03-0.125	≤0.03	≤0.03			
Fusobacterium sp. $(n = 11)$	≤0.03-64	0.5	1.0	≤0.03-0.5	0.06	0.125			

 $MIC_{50} = 50\%$ minimal inhibitory concentration; $MIC_{90} = 90\%$ minimal inhibitory concentration.

culture and the posttreatment culture from the moxalactam patient was contaminated with yeast, which precluded the ability to isolate C. trachomatis. One moxalactam-treated patient, who did not have a pretreatment culture obtained for chlamydia, had a posttreatment chlamydia-positive endometrial culture.

Bacteremia was noted in four of 44 study patients from whom blood cultures were obtained. The organisms, one from the blood of each of the four patients, included E. coli in a clindamycin/tobramycin-treated patient with postpartum endomyometritis and anaerobic Streptococcus sp., E. coli, and an unidentified anaerobic gram-positive coccus, each in a moxalactamtreated patient with salpingitis. The E. coli from the clindamycin/tobramycin-treated patient and the anaerobic Streptococcus sp. from the moxalactam-treated patient were isolated from pretreatment endometrial cultures from these patients. The moxalactam-treated patient with E. coli also had >100,000 colonies per milliliter of E. coli isolated from the urine but no E. coli from the endometrial culture. One additional patient had one of two blood cultures positive for two kinds of viridans streptozocci, which were considered to be contaminants.

Comment

The selection of antibiotic therapy for gynecologic and obstetric patients with pelvic infections must be based on the knowledge of microorganisms involved in these infections. Results of cultures taken prior to treatment from patients in this study continue to indicate that upper genital tract infections involve a complex microflora including N. gonorrhoeae, facultative organisms, especially enterobacteriaceae and streptococci, and anaerobic bacteria, especially peptostreptococci, peptococci, and Bacteroides, especially B. bivius.

Physicians involved in the treatment of pelvic infections should be aware of the emergence of B. bivius as a frequent pathogen in the female genital tract. This organism has been recovered from 19% to 52% of pa-

^{*}The minimal inhibitory concentrations of tobramycin were not determined for anaerobic organisms.

[†]Number of isolates.

Table VA. Microorganisms isolated from posttreatment cultures of 32 treated women

	No. of isolates							
Aerobic organism	Mo_alactam _Е тоиф	Clindamycin/ tobramycin group	Total	From primary plates				
Gram-positive cocci								
Group B streptococci	I	1	2	0				
Enterococci	3	4	$\bar{7}$	6				
Group D streptococci, not entero- cocci	-1	. 0	1	1				
α-Hemolytic streptococci	2	1	3	1				
Nonhemolytic streptococci	2	0	2	ì				
Coagulase-negative staphylococci	2	7	. 9	7				
Gram-positive rods								
Lactobacilli	6	5 .	11	7				
Diphtheroids	2.	$\dot{2}$	· 4	3				
Gram-negative rods		•						
Escherichia coli	0 ·	5	5	3				
Klebsiella pneumoniae	0	1	1	1				
Gardnerella vaginalis	2	2	4	2				
Unidentified coryneform organism	1	0	1	1				
Yeast				•				
Candida albicans	3	1	4	4				
Candida tropicalis	. 0	1	1	1				
Torulopsis glabrata	2	1	3	3				
Saccharomyces cervisiae	1	0	1	I				
Total	28	31	59	42				

Table VB. Microorganisms isolated from posttrea ment cultures of 32 treated women

	No. of isolates							
Anaerobic organism	Mozilectam Eoup	Clindamycin/ tobramycin group	Total	From primary plates				
Gram-positive cocci			•					
Peptococcus asaccharolyticus	0	1	1	0				
Peptococcus prevotii	1	0	1	1				
Peptococcus magnus	1	2	3 -	2				
Peptostreptococcus anaerobius	1	0	1	1				
Anaerobic Streptococcus sp.	1	0	1	1				
Unidentified anaerobic gram-positive cocci	3	1	4	1				
Gram-positive rods'		;						
Lactobacillus sp.	2	0	2	1				
Actinomyces sp.	1	0	1	1				
Propionibacterium sp.	Ι.	. 2	3	0				
Unidentified anaerobic gram-positive nonsporing rods	1 .	1	2	1 ′				
Gram-negative rods		•						
Bacteroides bivius	2	0	2	2				
Bacteroides fragilis group	2	. 0	2	1				
Bacteroides asaccharolyticus	1	0	1	1				
Bacteroides sp.	3	1	4	2				
Bacteroides splanchnicus	0	1	1	0				
Total	.0	, 9	29	15				

tients with pelvic infections.^{2,5,6} It is recovered far more commonly than the *B. fragilis* group (4% to 16%), ^{2,5,6} probably because it is more often a part of the endogenous cervical/vaginal flora than is *B. fragilis. Bactero Les disiens*, an organism similar to *B. bivius*, has also been isolated from genital tract infections.^{2,5,10}

The importance of recognizing B. bivius and B. disens in pelvic infections lies in the fact that they, similar to

B. fragilis, are often resistant to penicillin G, ampicillin, and first-generation cephalosporins, 9. 10 which have often been used as primary treatment in cases of pelvic infection. When patients did not respond to such regimens and did not require surgical intervention, clindamycin and chloramphenicol were added to provide coverage against Bacteroides. However, investigators using animal models11 and those performing clinical

Table VI. Minimal inhibitory concentrations for bacteria from post_reatment cultures

Organism and antibiotic group	Moxalactam			Clindamycin*			Tobramycin*		
	Range	MIC ₅₀	MIC ₉₀	Range	MIC ₅₀	MIC ₉₀	Range	MIC ₅₀	MIC ₉₀
Group B streptococcus Moxalactam (n = 1†) Clindamycin/tobramycin (n = 1)	<u></u>	4 8	4 8			•	***************************************	64 32	64 32
Enterococci Moxalactam (n = 3) Clindamycin/tobramycin (n = 4)		>128 >128	>128 >128				2-8 8-16	8 16	8 16
Escherichia coli Moxalactam (n = 0) Clindamycin/tobramycin (n = 5) Peptococcus asaccharolyticus	≤0.03-0.125	0.06	0.125				0.5-1.0	1.0	1.0
Moxalactam (n = 0) Clindamycin/tobramycin (n = 1)	-	0.125	0.125	_	>128	>128			
Peptococcus prevotii Moxalactam (n = 1) Clindamycin/tobramycin (n = 0)		1.0	1.0	_	≤0.03	≤0.03			
Peptococcus magnus Moxalactam (n = 0) Clindamycin/tobramycin (n = 2)		1.0	1.0	_	>128	>128			,.
Peptostreptococcus anaerobius Moxalactam (n = 1) Clindamycin/tobramycin (n = 0)		2	2	_	≤0.03	≤0.03			,
Unidentified anaerobic gram-positive cocci Moxalactam (n = 2) Clindamycin/tobramycin (n = 1)	2-128	2 2	128 2	0.06-0.25	0.06 128	0.25 128			
Bacteroides bivius Moxalactam (n = 2) Clindamycin/tobramycin (n = 0)	8-16	8	16	_	≤0.03	≤0.03			
Bacteroides fragilis group Moxalactam (n = 2) Clindamycin/tobramycin (n = 0)		>128	>128	0.5-1.0	0.5	1.0			

^{*}The minimal inhibitory concentrations of tobramycin were not determined for anaerobic organisms. The minimal inhibitory concentrations for clindamycin were not determined for aerobic organisms.

studies1, 12 have demonstrated that early intervention with either combination or single antimicrobial agents that are active against both facultative and anaerobic bacteria (especially Bacteroides) results in higher clinical cure rates and, more important, reduces the incidence of serious infectious complications such as bacteremia, abscess formation, and septic pelvic thrombophlebitis. Studies of pelvic infection in which antibiotic(s) effective against resistant Bacteroides species were used reported cure rates from 87% to 100% and severe infection incidences of 0% to 15%.1.12 In contradistinction, antimicrobial therapy that did not provide activity against the resistant Bacteroides resulted in lower cure rates, ranging from 70% to 90%, and, most significantly, a higher incidence of severe infectious compli-

catiors, ranging from 5% to 29%.1.12 As shown in this study and others, 4-6 moxalactam provides coverage against the organisms most commonly isolated from obsterric and gynecologic infections including group B strepiococci, N. gonorrhoeae, E. coli, peptococci, and B. bivius It was also active against the less commonly isolated B. fragilis group organisms in this study.

Although the response to moxalactam treatment was good in the current study, the number of anaerobic isolates in posttreatment cultures was fewer from patients who received clindamycin/tobramycin than from those who received moxalactam. This was especially true of Bacteroides isolates, of which there were eight from the posttreatment moxalactam group and only two from the posttreatment clindamycin/tobramycin

[†]Number of isolates.

group. Three of seven *Bacteroides* isolates from the moxalactam group and tested for susceptibility were resistant to moxalactam. No *Bacteroides* isolate was resistant to clindamycin.

Although fewer anaerobic organisms were isolated following clindamycin/tobramycin treatment, those that were were more likely to be resistant to clindamycin. Four of five isolates (from clindamycin/tobramycin—treated patients) that were tested (all anaerobic gram-positive cocci) were resistant to clindamycin. None of these was resistant to moxalactam. Only four (three *Bacteroides*, one gram-positive coccus) of 11 post-treatment isolates tested from moxalactam patients were resistant to moxalactam. None of these was resistant to clindamycin. None of the patients with resistant organisms in posttreatment cultures experienced adverse clinical effects.

The significance of the recovery of *G. vaginalis* from 68% of the pretreatment cultures is unknown. Recently several other investigators have reported isolating this organism as part of a mixed flora from infection site cultures of women with upper genital tract infectiors. Although we did not test the susceptibility of this organism to the study drugs, the absence of the organism from most posttreatment cultures suggests that it was susceptible to either drug regimen.

In the majority of patients with acute salpingitis, the endometrial cavity was the source of the specimens for culture. These endometrial specimens were obtained through the use of a protected sterile plastic cannula, which minimized vaginal and/or cervical contamination. In preliminary work, we have demonstrated that endometrial isolates more closely mirror fallopian tube isolates than do isolates from the cul-de-sac obtained by transvaginal culdocentesis. (Sweet RL, unpublished data). However, it is important to recognize that the optimal culture sites in patients with acute salpingitis are the fallopian tubes at the time of laparoscopy.

Toxic and allergic side effects associated with moxalactam therapy were minimal in this group of study patients. However, moxalactam use has been associated with prolonged bleeding time, probably secondary to reduced prothrombin synthesis. It has been postulated that this reduced prothrombin synthesis is related to elimination in the gut of vitamin K-producing bacteria. Not only moxalactam but other new cephalosporins that are also excreted via the bile (for instance, cefoperazone) could produce similar findings. A second possible explanation for the prolonged bleeding time is reduced liver synthesis of prothrombin related to the methyltetrazolethio side chain in some cephalosporin molecules, for instance, moxalactam, cefamandole, and cefoperazone. Use of vitamin K has been demonstrated to prevent these coagulation abnormalities; thus it has been recommended that vitamin K be given concurrently with moxalactam on a weekly basis. 13-16 Although bleeding times were not determined on patients in this study, clinical signs of prolonged bleeding were not evident in any patient.

Of increasing concern to clinicians and others involved in health care is the cost of this medical care. Thus, in comparative studies of antimicrobial regimens, cost of each drug regimen should be determined, especially when efficacy and safety of the drugs are similar. Therefore, we have calculated the cost of a 5-day course of moxalactam, 2 gm intravenously at 8-hour intervals, and clindamycin, 600 mg intravenously at 8hour intervals. The costs, which include charges for the drugs, intravenous infusion setup, and pharmacy preparation, were calculated for San Francisco General Hospital (a city-county hospital) and Children's Hospital in San Francisco (a private hospital). At San Francisco General Hospital a 5-day course of moxalactam costs \$322.08 while the clindamycin/tobramycin regimen costs \$300.68 (clindamycin, \$225.80; tobramycin, \$74.88). For Children's Hospital a 5-day regimen of moxalactam costs \$661.15 compared to \$909.48 for a 5-day course of clindamycin (\$675.60) plus tobramycin (\$233.88).

In conclusion, in our clinical setting and with our patient population, moxalactam in a dosage of 2 gm intravenously every 8 hours demonstrated excellent clinical efficacy with minimal side effects as a single agent in the therapy of mixed aerobic-anaerobic pelvic infections of a moderate to severe nature. Furthermore, our data on the occurrence of clindamycin-resistant anaerobes from cultures taken following clindamycin therapy suggest that clinicians may need to consider the possibility of clindamycin-resistant anaerobes in their own patients and thus be aware of alternatives to clindamycin therapy. However, with moxalactam the clinician must be alert to the potential for clinical failures due to moxalactam-resistant strains of B. fragilis, for superinfection by the enterococcus, an organism resistant to moxalactam, and for prolonged bleeding times (if vitamin K is not given concurrently with moxalactam). In addition, current Food and Drug Administration recommendations advise that the moxalactam dosage be no more than 4 gm/day to decrease the risk for bleeding problems. While this study and others have demonstrated the efficacy of 6 gm/day of maxalactam, Cunningham et al.4 reported the occurrence of a significant number of failures when a 3 gm/ day regimen of moxalactam was used in the treatment of post-cesarean section endomyometritis. Thus additional studies will be required to determine the efficacy of a 4 gm/day dose of moxalactam.

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Factors affecting the incidence of infectious morbidity after radical hysterectomy

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A double-blind, placebo-controlled trial was performed to assess the value of cefoxitin for prophylaxis against postoperative infection following radical hysterectomy. Infectious mcrbidity was observed in 35% of 43 patients in the control group and 23% of 31 in the cefoxitin group. In seven control patients (16%) and one patient (3%) in the cefoxitin group the infections were related to the surgical site (p = 0.07). These differences did not achieve statistical significance. Examination of the data "evealed a number of other factors, including operating time, patient weight, blood loss, and blood replacement, that were significantly related to the incidence of infectious morbidity. Comparison of the results of the present study with those in the literature indicates that a careful examination of the circumstances prevailing in any particular institution is necessary before a decision is made on strategies to combat infectious morbidity after radical hysterectomy. (AM J OBSTET GYNECOL 1985;152:817-21.)

Key words: Radical hysterectomy, morbidity, prophylactic æfoxitin

Among guidelines proposed by Ledger et al. to prevent the injudicious use of prophylactic antibiotics in

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Reprint requests: Denis Cavanagl, M.D., Department of Obstetrics and Gynecology, Box 18, University of South Florida College of Medicine, Tampa, FL 33612. gynecologic surgery were the requirements that the operation should carry a significant risk of operative site infection and should cause significant bacterial contamination. Radical hysterectomy would appear to be most appropriate for antibiotic prophylaxis. A recent survey of gynecologic oncologists indicated that 65% of the respondents routinely utilized prophylactic antibiotics for patients undergoing radical hysterectomy. Despite a number of publications attesting to their effectiveness in other gynecologic procedures, only two controlled studies have been reported that relate to the use of prophylactic antibiotics in radical hysterectomy. 2.6

We conducted a double-blind, controlled trial comparing the effectiveness of cefoxitin (Mefoxin, Merck Sharp & Dohme) with placebo in patients undergoing radical hysterectomy. The results offer insight into the variables affecting the incidence of infectious morpidity after this procedure and the factors involved in ceciding whether to use antibiotic prophylaxis.

Material and methods

The study involved 100 women admitted to Tampa General Hospital for radical hysterectomy by the Division of Gynecologic Oncology of the University of South Florida. Patients receiving antibiotic therapy within 72 hours of the planned operation and hose with clinical or laboratory evidence of infection or a temperature >38° C within 24 hours of the proposed operation were not asked to participate. Those with known allergy to cephalosporin or cephamycin antibiotics were also excluded.

All patients signed an informed consent form approved by the institutional review boards of bot L the hospital and the University of South Florida.

Preoperative investigations included a complete blood count with differential white blood cell court, an SMAC biochemical profile, and microscopic examination of the urine with culture and sensitivities. Chest roentgenography, intravenous pyelography, and barium enemas were performed the day prior to operation. Bowel preparation began following the radio ogic tests and was confined to a liquid diet and enemas antil clear but excluded antibiotics.

All patients received prophylactic heparin in a Hose of 3000 units every 8 hours from the evening pricr to operation until fully ambulatory.

Patients received "study drug" from identical kits with 12 ampules, each containing either 2 gm of cefoxitin or placebo according to a randomization performed prior to the study. The "study drug" was administered intravenously at 8-hour intervals, commencing approximately 16 hours prior to the scheduled operation, with the third preoperative dose being given 1/2 to 1 hour before operation. Following operation line doses were given at 8-hour intervals. The code was not broken until the entire study was completed.

Surgery was performed by a gynecologic oncologist and a fellow. The abdomen and vagina were prepæed with Betadine; a Foley catheter was inserted, and the abdomen was draped. A midline incision was used. After peritoneal lavage with 300 ml of normal saline-solution for cytologic examination, the abdomen vas thoroughly explored. If there was no evidence of extrapelvic disease para-aortic lymphadenectomy vas performed, with frozen section examination of the excised tissue. If results of this examination were negative a complete bilateral pelvic lymphadenectomy was car-

ried out to remove the common, external, and internal iliac nodes and the tissue in the obturator fossa above the level of the obturator nerve. Radical hysterectomy was then performed, with removal of the upper one third of the vagina and the parametrial tissues to the lateral pelvic wall. The vagina was closed around a Ttube drain attached to low-pressure suction. The distal ureters were suspended to perivesical fat and the obliterated hypogastric arteries. Hemovac drains were placed along each pelvic sidewall before reperitonealization and exited through the anterior abdominal wall bilaterally to be connected to low-pressure suction. Appendectomy was performed if the appendix was present. The abdomen was closed by a continuous massclosure technique with No. 2 Prolene sutures, and the skin was closed with staples.7

For postoperative febrile morbidity, defined as two consecutive oral temperatures of ≥38° C 4 hours apart, a workup was performed including thorough physical examination, pelvic examination, and bacteriologic studies of the urine and any other areas suspected of being infected. Blood cultures and a chest x-ray film were also obtained. The "study drug" was then stopped, and antibiotic therapy commenced with drugs other than cephalosporins. For suspected surgical site infections triple therapy with ticarcillin, gentamicin, and clindamycin was generally used. For non-surgical site infections a single agent was chosen based on bacterial sensitivity testing of either the cultured organism or the most commonly encountered sensitivity pattern of the suspected pathogens in the hospital. In patients in whom non-surgical site infection had been excluded, the infection was considered to be a pelvic soft tissue infection and treated as such.

In all patients the indwelling Foley catheter was maintained for 6 weeks. On discharge from hospital, patients were placed on nitrofurantoin, 100 mg three times per day, for urinary tract prophylaxis.

The distribution of each clinical measurement was assessed graphically and with measures of central tendency and variability. All clinical measurements were compared between treatment groups with Student's t test or one-way analysis of variance for continuous measurements and χ^2 tests for dichotomous measurements. The cumulative incidence of infection, length of operation, and duration of hospital stay were analyzed by the Product Limit method of Kaplan and Meier. The cumulative experience of subgroups over time was compared by a log rank method. Infection risk estimates were also assessed in a multivariant fashion by means of logistic regression analysis.

Results

One hundred patients were enrolled between June, 1980, and June, 1982. Of these, 51 received placebo

Table I. Basic characteristics of study groups

Characteristic	Study		
	$\begin{array}{c} Control\\ (n=31) \end{array}$	Cefoxitin (n = 43)	Overall $(n = 74)$
Mean age (yr)	48.1 (SD = 13.6)	50.7 (SD = 13.6)	49.2 (SD = 13.6)
Mean weight (kg)	68.4 (SD = 19.5)	68.9 (SD = 19.4)	68.6 (SD = 19.5)
Mean height (cm)	162.1 (SD = 7.1)	$^{-63.7}$ (SD = 6.3)	162.8 (SD = 6.8)

There was no significant difference between groups in terms of these factors

Table II. Surgical characteristics of the control and cefoxitin groups

	Study			
Characteristic	$Control \\ (n = 31)$	Cefoxitin (n = 43)	Overall $(n = 74)$	
Mean operating time (hr)	3.6* (SD = 1.2)	. 3.1* (SD = 1.0)	3.4 (SD = 1.1)	
Mean blood loss (ml)	832.6 (SD = 435.7)	765.7 (SD = 554.2)	794.2 (SD = 491.0)	
Appendectomy done	9	10	19	

^{*}Difference significant at p = 0.04 level.

and 49 cefoxitin. A total of 26 patients were excluded from analysis, 22 because the findings at laparotomy precluded radical hysterectomy, one because the patient was inadvertently given antibiotics after operation for urinary tract prophylaxis, and three because the correct drug dosage schedule was not adhered to. The number of patients finally analyzed was 74, 43 in the placebo group and 31 in the cefoxitin group.

Patient characteristics. The mean age of study patients was 49.2 years, the mean weight 68.6 kg, and the mean height 162.8 cm. There were no statistically significant differences in ages, weights, and heights between the control group and the cefoxitin group (Table I).

Surgical variables. The median operating time for the series was 3.4 hours; for the control group it was 3.6 hours and for the cefoxitin group 2.9 hours (p = 0.03). There is no apparent explanation for this difference. There was no significant difference in the incidence of appendectomy or the estimated blood loss between groups (Table II).

In the study group as a whole there were statistically significant relationships between increased operating time and increasing weight (p = 0.04), estimated blood loss (p = 0.0002), blood replacement (p < 0.0001), and the total incidence of infectious morbidity (p = 0.03).

Infectious morbidity. Surgical site-related infections were seen in eight patients (10.8%), and non-siterelated infections in 14 (18.9%). No patient had both. Of the non-site-related infections, 11 (78.6%) proved

Table III. Incidence of surgical site-related and non-site-related infections in study

	Study		
Site of infection	$\begin{array}{c} Control \\ (n = 31) \end{array}$	Cefoxitin $(n = 43)$	Overall $(n = 74)$
Surgical	7*	1*	8
Nonsurgical	8	6	14
Total infect ons	15	7	22

p = 0.07.

to be urinary tract infections. The remainder were respiratory infections, two with unknown pathogens, and in one Kle'ssiella pneumoniae was identified.

At 7 days following the operation the cumulative incidences of total infections and surgical site-related infections were 25% and 12%, respectively. By the time of discharge the total incidence of infection was 15 of 43 (35%) in the control group compared with seven of 31 (23%) in the cefoxitin group. This difference was not statistically significant. The major difference between the control and cefoxitin groups was the incidence of surgical site-related infection. This occurred in seven of 43 (16%) of the control group and in only one of 31 (3%) of the cefoxitin group. Again, this difference did not achieve statistical significance (p = 0.07) (Table III). The presumed site of infection is shown ir. Table IV. When significant febrile morbidity occurred and no source could be identified it was as-

Table IV. Actual site of infections in control and refoxitin groups

	Study		
Type of infection	Ca.trol (n = 31)	Cefoxitin $(n = 43)$	Overall $(n = 74)$
Surgical site-related	-		
Wound	ۓ	0	4
Fever, unknown site*	3	1	. 4
Non-site-related			
Urinary tract	7	4	11
Pulmonary	I	2	3

^{*}Where significant febrile morbidity occurred and all other sites were excluded, infection was assumed to arise from pelvic soft tissue.

Table V. Organisms obtained from various sites where cultures were positive

	Study			
Site	. Control (n = 31,	Cefoxitin (n = 43;	Overall (n = 74)	
Wound				
Staphylococcus	2	0	2	
Streptococcus	1	0	1	
Enterococcus	1	0	1	
Urine				
Escherichia coli	3	1	4	
Enterobacter	1	2	3	
Klebsiella	2	1	3	
Serratia	1	1	2	
Proteus	0	I	1	
Lung				
Klebsiella	0	1	1	

Several infections grew multiple organisms, while five wound infections produced no significant growth.

sumed to be surgical site-related, representing pelvic soft tissue infection.

The organisms isolated in cultures obtained from infected patients are listed in Table V. In two patients with urinary tract infections, one in each group, several pathogens grew; this was also true in one patient with wound infection in the control group.

Analysis of the study group as a whole showed significant relationships between the amount of blood replacement and the incidence of both non–surgical site related infection (p = 0.05) and total infectious morbidity (p = 0.05).

Hospital stay. The median length of hospitalization following operation was 12 days and did not differ significantly between the control (12 days) and cefoxitin (12 days) groups.

Hospital stay was significantly related to the incidence of surgical site-related infection (p = 0.02), non-surgical site related infection (p = 0.01), and the occurence of any form of infectious morbidity (p = 0.0001). The median duration of hospitalization in patients experiencing postoperative infection was 14 days compared to 11 days in patients without this complication (p = 0.0003).

Adverse reactions. There were no adverse reactions to either cefoxitin or placebo in the study.

Mortality. A 58-year-old patient weighing 98 kg, who was in the cefoxitin group and who had a proved urinary tract infection, developed clinical evidence of deep venous thrombosis on the fourteenth postoperative day. Prophylactic heparinization was changed to a therapeutic regimen, but despite apparently adequate anticoagulation she suffered a major pulmonary embolism on the sixteenth postoperative day and died.

Comment

This study was undertaken to investigate the efficacy of cefoxitin as a prophylactic antibiotic for patients undergoing radical hysterectomy. The overall incidence of infectious morbidity was lower in the group treated with cefoxitin (23%) than in the placebo group (35%) because of a reduced incidence of surgical site—related infection in the cefoxitin group (3%) compared to the placebo group (16%). These findings did not achieve statistical significance, a finding that may be due to the low power of the statistical tests used with the available sample size. Power analysis suggests that if an effect on the overall infectious morbidity of the magnitude ob-

served here exists, there was less than a 20% chance of a statistically significant result with the number of patients available. It was estimated that approximately 60 subjects would be required in each group to have a 90% probability of finding a significant reduction in surgical site infections at the observed level with a probability of a false positive result of ≤ 0.05 .

The study Rosenshein et al.6 of women given a single dose of doxycycline before operation failed to show a atistically significant decrease in the total incidence of surgical site-related infections. However, they did show a significantly lower "fever index." This was determined by measuring the area under the temperature curve with a baseline of 37° C during periods of 7 and 14 days. We believe that such a technique is not valid for the present study as treatment with alternative antibiotics was started immediately the criteria for febrile morbidity were met. Therefore, the "fever index" would reflect more on the effectiveness of the therapeutic antibiotics than on the prophylactic regimen.

In the study by Sevin et al.,2 using a protocol similar to that described in this paper, patients in the cefoxitin group had a significantly lower incidence of total, surgical site-related, and non-surgical site related infectious morbidity when compared with the placebo group. It is of note that the rates of infection reported in their study were considerably higher than those in the present study. They reported surgical site-related morbidity in 12.5% of the cefoxitin group and 48.1% of the placebo group. The total incidences of febrile morbidity were 41.7% for the cefoxitin group and 88.9% for the placebo group.

The divergence of results between the present study and that of Sevin et al. may be explained by examining factors found to be interrelated in our study. These include operating time, weight, estimated blood loss, blood replacement, and overall incidence of infection. Although in the present study the use of cefoxitin did not produce a significant change in the incidence of infectious morbidity, it would seem likely that a group of high-risk patients may benefit more noticeably from the use of prophylactic antibiotics.

Such a conclusion is in accord with that of Hemsell et al.,5 who performed a study with the use of cefoxitin in a protocol similar to that used in the present study

for women undergoing routine abdominal hysterectomy. They concluded that although prophylactic antibiotics were beneficial in their clinical setting, use in other institutions should be determined in accordance with the risk of infection in the particular setting. This is in agreement with the findings of a recent study of antibiotic prophylaxis for colorectal surgical procedures, which emphasized that the risk of infection in the patients managed by particular surgeons was an important factor to be considered in assessing the need for and effectiveness of prophylactic antibiotics.8

There is no doubt that the significant relationship between infectious morbidity and hospital stay, together with the high cost for the investigation and treatment of infectious morbidity, justifies careful research into methods of reducing this complication. It is apparent, however, that many factors other than the use of antibiotic prophylaxis are of critical importance in determining the incidence of infectious morbidity after radical hysterectomy. This underscores the need for individuals and institutions to assess their own particular situation in formulating approaches to the problem.

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Predictive value, sensitivity, and specificity of ultrasonic targeted imaging for fetal anomalies in gravid women at high risk for birth defects

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In this report the predictive value of ultrasonic targeted maging for fetal anomalies (TIFFA) is defined. Six hundred fifteen pregnant women at high risk for birth deects were scanned from January, 1980, to December, 1983. Follow-up evaluation was available on-569 fetuses. The pregnancies were classified into five groups according to the indications used for ultrasocic targeted imaging studies. The largest number of women were placed in group 1 and were referred because of a variety of abnormalities in previous or ongoing pregnancies. The women classified in the other four groups were examined because of maternal or fetal reasons related to specific craniospinal (29%), ut nary (7.9%), gastrointestinal (6.7%), and skeletal (3.7%) defects. In our series the predictive values of abrormal and normal ultrasonic targeted imaging studies were 95% and 99%, respectively. A detailed breakdown of the accuracy of ultrasonic targeted imaging in relation to each anatomic category is presented; these data are useful in counseling gravid women with anomalous fetuses. (AM J OBSTET GYNECOL 1985;152:822-7.)

Key words: Accuracy of ultrasound, fetal anomaLes

Detailed ultrasonic targeted imaging for fetal anomalies (TIFFA) or Stage II ultrasound is not routhely performed in most of the diagnostic ultrasound centers in the United States. Its use is basically intended for pregnant women at high risk for birth defects. The reasons are as follows: The cost-to-benefit ratio of noutine use is not established; prolonged exposure to ultrasound for targeted imaging examinations is deemed unnecessary in the face of a low yield of anomalies in normal pregnancy; the accuracy of targeted ultrasonic imaging is not fully established.

This study is designed to determine the predictive value of targeted ultrasonic imaging examination to: (1) enable us to better counsel patients referred for this specialized procedure and (2) serve as a basis for omparison with the findings of other, similar departments.

Patients and methods

We conducted a prospective study from January, 1980, to December, 1983, to compare the ultrasenic results with those of the neonates.

Six hundred fifteen pregnant women at high risk-for anomalies had an ultrasonic examination in the fcrm.

of detailed targeted imaging for fetal anomalies. They were referred from a large pool including the Northwestern Perinatal Center as well as primary providers of care. The referrals represented a predominantly midwestern population of middle socioeconomic level but of different ethnic backgrounds.

During the period of the study the number of referrals increased sharply from 162 in 1980 and 1981 to 453 in 1983 and 1984. Information on outcome was obtained by direct correspondence with the parents or by direct telephone communication with either the parents or the pediatrician(s) involved. Outcomes were available in 596 pregnancies, which form the data base of this report.

Patients were classified into five groups according to the indications used for initiating ultrasonic targeted imaging studies. Three hundred fourteen patients were classified in group 1. They were referred for a variety of abnormalities including: (1) high level of α -fetoprotein in amniotic fluid; (2) suspicion of abnormality on a standard or basic (Stage I) scan, including polyhydramnios and oligohydramnios; (3) fetus presenting as breech at term; (4) intrauterine growth retardation; (5) insulin-dependent maternal diabetes mellitus; (6) other reasons, such as exposure to teratogenic agents, preceding cervical cerclage, balanced translocations, lack of family medical history due to adoption, and anxiety.

Additionally, we included women at high risk for fetal congenital heart disease in group 1. However, in these patients ultrasonic targeted imaging examina-

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Table I. Indications and results of ultrasonic targeted imaging studies performed on pregnant women at high risk for anomalies involving the central nervous, genitourinary, gastrointestinal, and skeletal systems

		Diagr	osis by	Result of study					
	Positive	Basic	,	Correct diagnosis		False diagnosis			
Anomaly	family history	scan	Other	Normal	Abnormal	Normal	Abnormal		
Central nervous system									
Anencephaly	36	6	_	37	5		_		
Hydrocephalus	32	28	7	48	18	1			
Spina bifida	43		5*	41	5	1	1		
Encephalocele	9		1†	9	.1	1‡	_		
Holoprosencephaly	3		_	3	_		_		
Microcephaly	2	1.	_	2	1 .	****	_		
Total	125	35	13	140	30	2‡	1		
Genitourinary system									
Polycystic kidney	26			24	2	_			
Multicystic kidney		****	-						
Renal agenesis	4	18	- 18	4	1	1	_		
Ureteropelvic junction obstruction	2	2	_	2	. 1		1		
Ureterovesical obstruction	<u>~</u>	1 .	_		1				
Urethral obstruction	3	7	_	3	7¶		_		
Total	35	11	. 1	3	12	1	1		
Gastrointestinal system									
Duodenal atresia	_	2	_		2		_		
Gastrointestinal obstruction below	1	1	2#	_	$\overline{4}$	_			
duodenum									
Ómpḥalocele	19	9	1**	18	10	-	1		
Gastroschisis	1		_	1	_		_		
Diaphragmatic hernia	2	1	_	3	_				
Hirschsprung disease††	1		. —	would.	_		-		
Total	24	13	3	22	16		1		
Skeletal system									
Limb reduction defect	20		_	15	5	_	_		
Split hand syndrome	1		_		1		_		
Arthrogryposis‡‡	î		_		_	_	_		
Total	22			15	6				
i Qiai	44		_	10	U		_		

^{*}High α-fetoprotein level in four patients and hydrocephalus in one.

tions were directed only at the four-chamber anatomic structures to rule out primary and secondary atrial septal defects, single atrium or ventricle, hypoplastic ventricle(s), and large ventricular septal defect. Patients were informed that in utero diagnosis of heart defects was still in a developing phase and only information regarding the structural and dynamic components noted in the four-chamber view of the heart could be offered.

The remaining 282 pregnancies included women at high risk for specific anomalies involving the central nervous system (group 2), urinary system (group 3),

gastrointestinal system (group 4), and skeletal system (group 5). Classification in groups 2 through 5 was based on a positive history and suspicion of an anomaly on a basic scan (Table I).

Although scanning was done before 24 weeks of pregnancy in many patients, some were not referred until the third trimester. Additionally, in patients at high risk for anomalies that may not be apparent by 24 weeks' gestation (for instance, infantile polycystic kidney disease and heterozygous achondroplasia) repeat scans were specifically requested by 28 and 32 weeks of pregnancy. Our definition of "correct abnor-

[†]Hydrocephalus.

[‡]Same patient had false normal diagnosis of hydrocephalus and encephalocele.

[§]Oligohydramnios.

^{||}Resolution of hydronephrosis.

[¶]Mesoderm defect of muscle (two fetuses).

[#]Hydramnios.

^{**}High α-fetoprotein level.

^{††}Equivocal finding by ultrasound, deleted from results.

^{##}Mild disease, deleted from results.

Table II. The overall predictive value, sensitivity, and specificity of ultrasonic targeted imaging examinations performed on 594 pregnant women at high risk for congenital anomalies

· ·	Congenita	l anomaly	
Study results	Present	Absent	T əta
Abnormal	78	4	72
Normal .	. 3	509	5 2
Total ·	81	513	5 14*

Predictive value of abnormal examinations = 78 cf &2 or 95%. Predictive value of normal examinations = 50 & or 512 or 99%. Sensitivity = 78 of 82 or 95%. Specificity = 509 of 512 or 99%.

*Two fetuses with equivocal results (Hirschsprung di ease and arthrogryposis) were deleted from analysis of the total group, reducing the total number to 594 pregnancie.

mal" included: (1) Accurate ultrasonic diagnosis of the defect made prior to 24 weeks' gestation. The diagnosis was also considered accurate if the abnormality was not initially noted (for instance, at 18 weeks) but was subsequently visualized on a repeat scan that we scheduled. (2) The anomaly under consideration was visualized in the third trimester of pregnancy simply because the patient was not referred at an earlier cate.

These guidelines were also used for the definition of "correct normal." Incorrect diagnoses were classified as "false normal," "false abnormal," or "equivocal" (Table I).

The ultrasonic consultation for ultrasonic targeted imaging studies required an average of I hour and encompassed: (I) documentation of the history and reason for referral; (2) performance of a basicu trasound scan (Stage I), usually by a nonphysician \bar{t} refessional trained in obstetric and gynecologic sonography; and (3) detailed imaging of fetal structural aratomy from a variety of sagittal, transverse, corona, and oblique planes by a physician ultrasonograph r (sonologist).

Results

In the study population of 596 women, two extrases with equivocal results were deleted from the predictive value analysis. In the remaining group the predictive values of abnormal and normal targeted ultrasolic imaging studies (without reference to the prevalence of disease) were 95% and 99%, respectively (Table ID.

Group 1. The largest number of women (314 ⊃ f 594 or 53%) were classified in group 1. Of these pregrancies, 21 of 594 or 3.5% had polyhydramnios, diagnosed by the presence of amniotic fluid in excess of 7 tc 8 cm, measured in a plane vertical to the sagittal axis ⊃f the uterus, and six of 594 or 1% had oligohydramnios,

Table III. Abnormalities detected by targeted imaging for fetal anomalies in high-risk pregnant women with a variety of findings in previous or ongoing pregnancies not pertaining to the central nervous, urinary, gastrointestinal, or skeletal systems (group 1)

Abnormality	No.	Outcome
Polyhydramnios	21	Normal (16/21 or 76%) Tracheoesophageal fistula (1/21) Trisomy 18 (1/21) Endocardial fibroelas- tosis (1/21) Intestinal obstruction (2/21)*
Oligohydramnios	6	Severe growth retarda- tion (2/6) Spontaneous abortion (2/6)
		Renal agenesis (2/6)*
Cystic hygroma	6	Pregnancies terminated
Nonimmune hydrops	3	Fetal/neonatal death
Sacrococcygeal teratoma	2	Neonatal surgical procedure
Mass in chest	2	Cystic adenomatoid malformation (1) Iatrogenic pneumome- diastinum and false abnormal (1)
Chorioangioma	· 1	Premature labor
Ovarian mass	1	Neonatal surgical procedure (benign)
Total	42*	*
		•

*Of 42 positive findings; 20 were associated with amniotic fluid volume abnormalities and normal fetuses; of the remaining 22 fetuses, three fetuses had conditions not demonstrable by ultrasound, one fetus with a mass in the chest had a false abnormal result, one fetus with a false normal diagnosis of renal agenesis was counted in Table I, two fetuses with correct abnormal diagnoses of gastrointestinal obstruction and one fetus with a correct abnormal diagnosis of renal agenesis were counted in Table I, and 14 had correct abnormal diagnoses.

diagnosed by a paucity of amniotic fluid and overcrowding of fetal parts. The anomalies noted in this group are listed in Table III.

No anomalies were detected in 30 insulin-dependent diabetic gravid women. Similarly, no cardiac defects were noted in 20 patients at high risk for congenital heart disease.

The outcomes in four fetuses with chromosomal translocations were as suggested by ultrasound, normal. In eight pregnancies the amniotic fluid α -fetoprotein level was >3 SDs above the mean and the sonographic diagnoses were: spina bifida (five fetuses), omphalocele (one fetus), cystic hygroma (one fetus), and normal (one fetus).

Group 2: Central nervous system anomalies. One hundred seventy-three fetuses were scanned for possible craniospinal defects (Table I). In this series the

Table IV. Predictive value, sensitivity, and specificity of ultrasonic targeted imaging examinations in the diagnosis of spina bifida

0. 1	Spina		
Study result	Present	Absent	Total
Abnormal	5	l	6
Normal	1	41	42
Total	6	42	48

Predictive value of abnormal examinations = five of six or 83%. Predictive value of normal examinations = 41 of 42 or 97.6%. Sensitivity = five of six or 83%. Specificity = 41 of 42 or 97.6%.

predictive values of abnormal and normal results were 96.7% and 98.6%, respectively. In the subset of fetuses with spina bifida the predictive value of an abnormal result was lower than that of the group with craniospinal defects (Table IV).

Group 3: Urinary system anomalies. In fetuses examined sonographically for possible urinary abnormalities (Table I) the predictive values of abnormal and normal results were 92% and 97%, respectively. Two fetuses with abdominal muscle deficiency anomaly were incorrectly diagnosed as having urethral obstruction (see Comment section).

Group 4: Gastrointestinal system anomalies. In 40 high-risk pregnancies ultrasound studies were performed for possible fetal gastrointestinal anomalies (Table I). The predictive values of abnormal and normal results were 94% and 100%, respectively. In one fetus at high risk for Hirschsprung disease the ultrasonic findings were equivocal and the patient was excluded from the predictive value calculation.

Group 5: Skeletal system anomalies. Twenty-two fetuses were examined for the possibility of short limb dysplasia (Table I). A correct diagnosis was made in all six affected fetuses. The findings in one fetus at high risk for arthrogryposis were equivocal and the patient was excluded from the predictive value calculation.

Comment

In this series ultrasonic studies involving detailed targeted imaging for fetal anomalies were performed on 596 pregnant women at high risk for birth defects. Two fetuses were excluded because of equivocal results. In three fetuses the diagnosis could not have been made by ultrasound (Table III). In the remaining group there were 81 fetal anomalies (13.6%), four false abnormal results (0.6%), and three false normal results (0.5%). By comparison, in a large number of women examined ultrasonically by Campbell,1 17% had fetal anomalies and the rates of false abnormal and false normal results were 0.3% and 0.8%, respectively.



Fig. 1. Scan showing cross section of fetal trunk in sacral area. The iliac wings are shown by large arrows and the open spina bifida by small arrows. Neural tissue (two white echoes) is seen in the center of the defect. The bladder (b) is anterior.

Analysis of subsets in 173 fetuses at high risk for craniospinal defects (group 2) showed that all cases of anencephaly were correctly diagnosed, for a detection rate similar to that reported by other studies.2.3

Hydrocephalus was missed in one fetus who also had absence of skull bones anteriorly, encephalocele by definition. The fetus was examined only once and although the sonogram was suspicious in that ventricular dilation was borderline the report was signed out as normal.

Although a variety of craniospinal defects were suspected by basic scans, performed elsewhere, in 35 fetuses none of these diagnoses included spina bifida (Table I). The latter remains one of the most difficult abnormalities to diagnose even by ultrasonic targeted imaging examinations.3

The predictive value of abnormal ultrasonic targeted imaging examinations in the diagnosis of spina bifida was 83%; by contrast the specificity or predictive value of a normal result was 98% (Table IV). The latter statistic is particularly useful in counseling women with an elevation in the level of amniotic fluid α-fetoprotein, even when acetylcholinesterase is also elevated. The reason is that these biochemical tests are associated with false normal and false abnormal results. 3,4 In one report by Hobbins et al.,3 in 14 of 28 or 50% of pregnancies with elevated α-fetoprotein levels the offspring were devoid of overt anomalies.

The fetus in whom a spina bifida was missed in this study was also hydrocephalic and the amniotic fluid volume was very small. Because of oligohydramnios the scan was suboptimal. In the one fetus in whom a false abnormal diagnosis of spina bifida was made (Table IV)

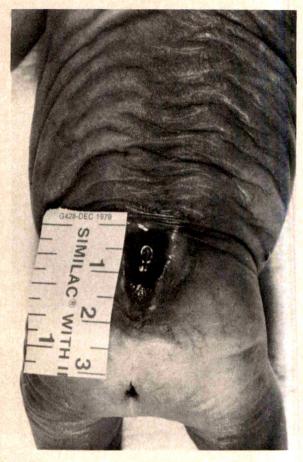


Fig. 2. Photograph of open spina bifida in the sacral area measuring 1.8 cm and visualized by ultrasound (see Fig. 1).

the scan was performed early in the series. Retrospective review showed that the image produced was slightly oblique resulting in an artifactual U-shaped or cuplike spine simulating a thoracolumbar spina bifida.

The sensitivity for detection of spina bifida in this series of 83% fell between those reported in two other studies, namely, 79% and 93%.^{1, 2} However, it should be appreciated that in these studies data were collected about 5 years prior to the publication of any reports when resolution of the ultrasonic equipment was less optimal and experience of sonologists in this area not extensive. Recently, with the equipment now available it was possible for one of us to visualize a spina bifida <2 cm in size and situated in the lumbosacral area (Figs. 1 and 2). Such small lesions were previously reported to be ultrasonically undetectable.²

In one fetus at high risk for infantile polycystic kidney disease, an autosomal recessive disorder, the anomaly did not manifest itself until after the twenty-fourth week of pregnancy.⁵ The earliest reported diagnosis of infantile polycystic kidney disease was at 18 weeks' gestation.⁶

In one pregnancy complicated by oligohydramnios renal agenesis was missed at term (Table I). Retro-

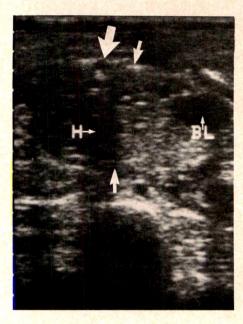


Fig. 3. Echogram showing cross section of fetal trunk in the lower chest area (H = heart). The spine is anterior (large arrow). The fetal bladder (BL) is dilated and appears outside the trunk (delineated by small arrows) as in omphalocele. The fetus, however, had an abdominal muscle deficiency anomaly in which mesoderm is lacking in the abdominal wall and urinary tract musculature.

spective review of the scans showed that kidney-like echoes were indeed noted paraspinally. Apparently at term such echogenicity can arise from fat deposition in the pararenal spaces⁷ and/or fetal adrenal glands.⁸ Thus in suspected cases of renal agenesis ultrasonic examination should be directed toward outlining the fetal bladder. Toward this end intravenous administration of furosemide (60 mg) to the mother may be considered.⁸ However, the beneficial effect of this approach particularly in the presence of intrauterine growth retardation is doubtful.⁹

A false abnormal diagnosis of unilateral ureteropelvic junction obstruction was made in one case; this was attributed to spontaneous resolution, a phenomenon reported by other investigators.¹⁰

A diagnosis of ureterovesical obstruction was made in two cases. Marked convolutions of the ureters differentiated this condition from that secondary to urethral obstruction. Both fetuses exhibited marked enlargement of the heart (without cardiac structural defects) and died in the neonatal period, despite successful in utero shunting.

Of interest is that two of seven patients referred for possible in utero shunts to drain a dilated urinary system (secondary to possible urethral obstruction) had an abdominal muscle deficiency anomaly (Fig. 3).¹¹ Garris et al.¹² described this disorder clearly and classified it as prune-belly syndrome, subgroup 3. Insertion of in

utero diversion shunts for this condition may not be necessary.

In two fetuses the diagnosis was made after 24 weeks' gestation. Nelson et al.¹³ doubt that this condition can be diagnosed prior to the twenty-fourth week of pregnancy and conjecture that the amniotic fluid swallowed will only exceed the resorptive capacity of the gut in the early part of the third trimester of pregnancy. The prenatal diagnosis of this condition is important, regardless of when it can be made, because it leads to investigation of the fetal karyotype and permits detailed planning for delivery at an optimal site as well as anticipatory aspiration of the gastric contents and rapid neonatal confirmation of the defect.¹⁴

In four fetuses with gastrointestinal obstruction below the duodenum, the correct diagnosis was made. Differentiation from simple distention, noted in some large-for-gestational age fetuses at term, was made by a marked increase in bowel size, thinning of the walls of dilated segments, and the presence of mild ascites.

The false abnormal diagnosis of omphalocele in one case (Table I) may have been the result of contraction of the abdominal musculature, which created an artifactual image of a small omphalocele. The differentiation between omphalocele and gastroschisis was described by Jassani et al. ¹⁴ It should be remembered that up to 50% of fetuses with omphalocele will have other associated abnormalities, particularly cardiac and chromosomal.

In the diagnosis of skeletal dysplasia (Table I) the accuracy of ultrasound was enhanced because five of six fetuses were severely affected and the anomaly readily manifested. In the fetus with heterozygous achondroplasia (a product of parents with the same condition) the diagnosis of limb shortening was not apparent at 16 weeks of pregnancy, but the femur length at 24 weeks was 3.5 cm, well below 2 SDs of the mean.

Detailed ultrasonic targeted imaging examinations in our center by experienced physician ultrasonographers failed to verify the presence of anomalies in 23 fetuses examined ultrasonically elsewhere. In these referrals false diagnoses were made of hydrocephalus (n=17), anencephaly (n=1), urethral obstruction (n=2), duodenal atresia (n=1), omphalocele (n=1), and diaphragmatic hernia (n=1). These observations attest

to the importance of properly conducted ultrasonic targeted imaging studies.

In this report the predictive values of abnormal and normal ultrasonic targeted imaging examinations were 95% and 99%, respectively. However, in the general population the accuracy will depend on: (1) expertise of the examiner; (2) prevalence of the anomaly in specific geographic locations; (3) whether the population undergoing examination was screened by history, biochemical tests, or basic ultrasound scans or for other pregnancy complications; and (4) period of follow-up, since subtle, nonlethal anomalies may be undetected early after birth.

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Behavioral states in the human fetus during labor

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Behavioral states in the near-term human fetus have been described during pregnancy. The aim of this study was to observe whether these same states are present during labor. Nine patients with uncomplicated singleton pregnancies participated. Fetal heart rate and uterine contractions were recorded. Fetal eye, mouth, rotation, and retroflexion of the head were observed by real-time ultrasound. Fetal movements were recorded with coded event-markers. Behavioral states were identified by the movement pattern. A total of 13 ultrasound observations, varying from 25 to 66 minutes, were obtained. Optimal viewing was present at least 60% and on the average 80% of the time. State 1F (quiet sleep), state 2F (active sleep), and state 3F (quiet awake), as well as a total of 10 state changes, were identified during labor in spite of increasing contractions and/or ruptured membranes. These observations demonstrate existence of alternating behavioral states in the healthy terms fetus during labor. (AM J OBSTET GYNECOL 1985;152:828-33.)

Key words: Fetus, behavioral states, labor, heart rate

Behavioral states in the neonate have been described by Prechtl¹ as being "distinct conditions, each having its specific properties and reflecting a particular mode of nervous function." Prechtl used only observable criteria (breathing, body movements, open/closed eyes, vocalization) and numbered the states 1 to 5 to prevent the danger of premature physiologic interpretation.

Nijhuis et al.² have demonstrated the presence of similar behavioral states during pregnancy in the near-term human fetus. The movements applied to recognize fetal behavioral states in their studies were eye, mouth, and body movements together with the heart rate pattern. Crying and breathing are obviously not possible to use as state criteria in the fetus. The fetal states are numbered 1F to 4F in a grouping similar to that of the neonate.

Van Geijn et al.³ observed that during pregnancy the near-term human fetus spends approximately 90% of the time in either quiet sleep (state 1F) or in active sleep (state 2F). During uninterrupted 2-hour observations with two real-time ultrasound transducers, one directed at the fetal head and the other at the fetal trunk, state 1F appeared to last 21 ± 6 minutes, whereas state 2F lasted 49 ± 22 minutes. Occasionally, states 3F (quiet awake) and 4F (active awake) were observed.

This paper will report on the existence and transition of behavioral states in the fetus during labor.

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Material and methods

Subjects. A group of nine patients (six multiparous and three primiparous women) with uncomplicated singleton pregnancies participated in this study. The women had an appropriate body weight and used no drugs except supplementary iron throughout pregnancy. The age of the mothers varied from 20 to 35 years. Two women smoked, although less than five cigarettes per day. All patients had participated in at least two antepartum recording sessions, each with a duration of 2 hours, beginning at 37 weeks of gestational age.

Criteria for onset of labor were regular uterine contractions at less than 5-minute intervals and a cervical dilation of at least 4 cm. Deliveries all took place within 11 hours following the first ultrasound observation. All of the infants were born in occiput anterior or lateral position. Each of the infants had a birth weight (2540 to 4365 gm) appropriate for the gestational age (38% to 42% weeks). Six girls and three boys were born. The Apgar score was 10 at 5 minutes for all infants.

The pH of the umbilical artery exceeded 7.23 in all cases except one (pH of No. 2 was 7.17) and once the measurement failed (No. 9) (see Table I). All infants had a normal neurological examination according to the technique described by Prechtl and Beintema.⁴

Methods. The women participating in this study were requested to arrive at the hospital at early onset of labor and were familiar with the registration techniques, having experienced at least two antepartum recordings.

Following the first examination of the patient, a long period of ultrasound observation commenced, together with simultaneous recording of the fetal heart rate and maternal contractions. As labor proceeded, often only shorter periods of ultrasound observation were ac-

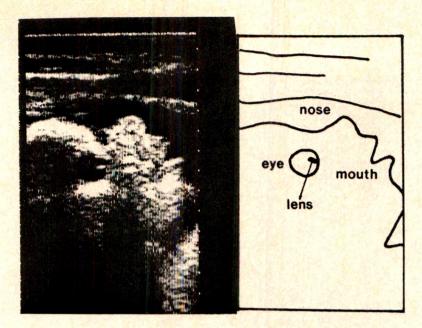


Fig. 1. Optimal ultrasound image (fetal head including one orbit and mouth).

Table I. Patient data

Case No.	Gravidity	Parity	Time of first stage labor (hr., min)	Time of second stage labor (min)	Amenorrhea (wk)	Sex of child	Birth weight (gm)	Apgar score (1 min/5 min)	pH of umbilical artery
1	3	2	5 hr, 10 min	7	39 wk, 3 days	Female	2540	9/10	7.24
2	2	1	5 hr, 00 min	16	39 wk, 2 days	Male	4365	8/10	7.17
3	2	- 0	9 hr, 15 min	62	38 wk, 6 days	Female	3960	9/10	7.29
4	3	2	11 hr, 00 min	28	42 wk, 3 days	Female	4110	8/10	7.28
5	2	1	4 hr, 40 min	56	39 wk, 5 days	Female	3510	10/10	7.26
6	2	1	6 hr, 00 min	22	39 wk, 5 days	Male	3600	10/10	7.38
7	1	0	10 hr, 00 min	67	40 wk, 2 days	Male	3340	9/10	7.23
8	2	1	6 hr, 00 min	13	42 wk, 2 days	Female	3880	10/10	7.36
9	1	0	10 hr, 00 min	12	40 wk, 1 day	Female	2960	9/10	Failed

ceptable for the patient. The patient was encouraged to be in whatever position was comfortable. Between observations the patient sometimes moved around the delivery room or labor ward, since we did not want to interfere with the normal process of labor. All observations were made by the same examiner.

The delivery room used in all of the registrations was outfitted with a direct connection to the computer across the hall, facilitating simultaneous processing of the signals.5 The fetal heart rate was obtained by means of abdominal electrocardiography in six subjects and by a spiral scalp electrode determination in three subjects (No. 2b, No. 4b, and No. 7). Uterine contractions were recorded externally, except in two cases (No. 4b and No. 7). Fetal heart rate signals and uterine contractions were recorded with a cardiotocograph (Hewlett-Packard 8030A) on paper. The processed beat-tobeat R-R intervals and intrauterine pressure curve were fed directly into the computer (DEC PDP 11/34). The ultrasound images, obtained from a Toshiba SAL-20A

Table II. Behavioral state criteria

State	Fetal movements					
	Eye	Mouth	General body, head, limb			
1F	_	Regular				
2F	+	Irregular	+			
3F	+	i i				

+ = Present, - = absent.

real-time unit with application of a 8 cm, 3.5 MHz probe, were recorded on videotape for later processing:

In all of the observations the fetal head, including one orbit and the fetal mouth, was viewed (see Fig. 1). In this manner the fetal eye6 and mouth movements as well as rotation and retroflexion of the head could be observed. Occasionally general body and breathing movements in the upper thorax and movements of a limb could be seen. A clock projected on the video screen facilitated exact synchronization of the fetal

Table III. Observation data

The state of the s										
Patient observation No.	Total time observed (min)	% of time in good view	State 1F	State 2F	State 3F	State change	Dilation (cm)	Condition of membranes	Uterine contraction curve	Fetal heart rate
la	45	91	-	-			5	Intact	+	+
b	26	81	******	might.		***	8	Intact	+	+
2a	50	95	Ann	4		-	4	Ruptured	+	+
b	56	70	****		*****		7	Ruptured	+	+*
3	30	97	+	- <u>F</u>	_	+	4	Ruptured		+
4a	27	98	+	+	_	+	4	Ruptured	+	+
b	28	96	+	+		+	9	Ruptured	+†	+*
5	56	97	+	++		++	4	Ruptured	+	+
6a	66	86	+	+		+	4	Intact		+
b	31	61	+	+	- Arten	+	6-9	Intact		+
7	33	83	****	+			7-8	Ruptured	+ †	+*
8	25	73	+	+	N-46.	+	5	Intact	+	+
9	51	80		++	+	++	4	Ruptured		+

⁺ = Present, - = absent.

movements observed during later processing together with the fetal heart rate and uterine contractions, which had already been recorded on-line. Fetal movements were recorded by means of event-markers. Each of the seven observable movements was coded during several video-replay sessions, and the information was added to the information already in the computer. The push buttons were pressed when the observer recognized a particular movement and were held in this position as long as a movement occurred. When the movement stopped, the marker was released. A foot pedal was used to indicate optimal view of a fetal orbit.

Fetal behavioral states during labor were identified applying the criteria described in Table II. In our study the fetal heart rate pattern was not included as a criterion in the primary designation for a behavioral state or an alteration in state.

Results

Of these nine patients, a total of 13 observations were obtained (Table III). The fetal face was in good view at least 60% of the time in each of these 13 observations. In nine the optimal viewing time exceeded 80%. The length of the observation period varied from 25 to 66 minutes, with a mean duration of 40 ± 14 minutes. The maximum break in the observation of a fetal orbit was 10 minutes; however, breaks rarely exceeded more than a couple of minutes and then mostly during strang uterine contractions.

From the eye, head (body), and limb movements observed, fetal behavioral states could be recognized as being similar to those observed during pregnancy in the near-term fetus.^{2, 3}

A total of seven periods of state 1F was identified.

All of these recordings were either preceded or followed by state 2F. Enclosed epochs are episodes of a certain behavioral state, which are preceded and followed by another behavioral state. One period included a so-called enclosed epoch and lasted 23 minutes, with a state 2F observed immediately before and following the 1F period. During the 1F periods only very infrequently was an eye movement observed. General movements as seen in state 2F were completely absent. An occasional single, quick, high-velocity movement of the fetal head, limb, or trunk was observed, similar to the startle seen in state 1 (quiet sleep) in the newborn infant. Mouthing movements, indicated as jaw opening in our recordings, were seen as being regular in frequency and duration. Regular mouthing movements were seen in all nine of the fetuses and in six of the seven periods of state 1F. Fig. 2 is a typical example of state 1F with a change to 2F.

A total of 15 periods of state 2F was observed in all of the nine fetuses. There were no periods of enclosed epochs in state 2F. During 2F, fetal eye and mouth movements were recorded, as well as large fetal movements, including general body movements and rotation/retroflexion of the head and limb movements. Mouthing movements (jaw opening) were irregular in frequency and duration. Two periods of fetal breathing (No. 1a and No. 9) were also clearly visible. Fig. 3 is a characteristic example of a recording of a state 2F.

In one case (No. 9) a state 3F clearly was observed. This 3F recording lasted a total of 35 minutes and was an enclosed epoch being immediately preceded and followed by state 2F. During this time fetal eye movements were observed, but body, head and mouth movements were absent.

^{*}Scalp electrode.

[†]Intrauterine pressure.

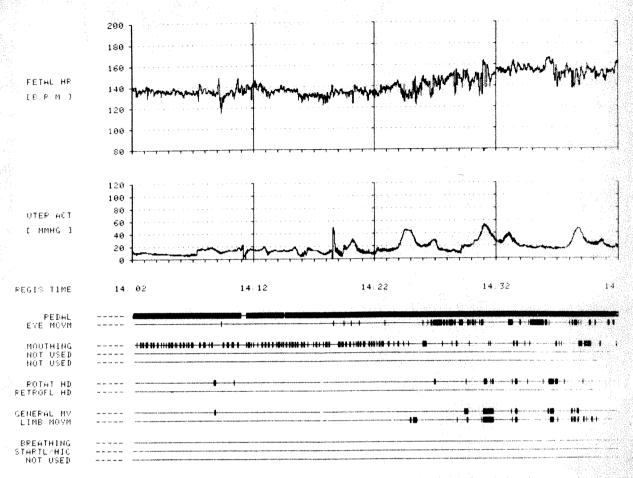


Fig. 2. Recording state 1F (quiet sleep) and a change to state 2F (active sleep) at 14 hours, 24 minutes (No. 5).

Only two enclosed epochs were fully observed during labor. This was most probably due to the fact that the ultrasound observations were relatively short in comparison with the behavioral state durations, because of the circumstances during labor.

In these nine fetuses a change from state 1F to 2F was observed five times (No. 3, No. 4a, No. 4b, No. 5, and No. 6a) and a change from 2F to 1F was observed two times (No. 5 and No. 6b). Changes from 2F to 3F and from 3F to 2F were also seen. Altogether a total of 10 changes in the behavioral state was recorded during the 13 periods of ultrasound observation. Even though the membranes had ruptured long before the observation took place, in four fetuses (No. 3, No. 4, No. 5, and No. 9) a change in state was observed, whereas in two other fetuses only a state 2F was seen.

Although the fetal heart rate was not considered as a criterion as the states were designated, it is interesting to see that periods of low fetal heart rate variability, when there are no fetal movements, do correspond with states 1F or 3F as described by Nijhuis² and van Geijn et al.3 Periods of high fetal heart rate variability and periodic accelerations in the fetal heart rate did cor-

respond to state 2F. In several cases (No. 2, No. 3, No. 5, and No. 7) accelerations in the fetal heart rate clearly corresponded with large fetal movements and could be observed between uterine contractions. During periods of low fetal heart rate variability, as seen in state IF or quiet sleep, even very strong uterine contractions 30 minutes prior to delivery did not affect the fetal heart variability.

Comment

Fetal behavioral states as recognized during pregnancy in the near-term human fetus are also present during labor. Under normal circumstances the fetus continues his own behavior state patterns, changing periodically from quiet (state 1F) to active (state 2F) sleep states or vice versa, despite increasing frequency and strength of maternal uterine contractions or the existence of ruptured membranes. A period of low fetal heart rate variability, that is, absence of heart rate accelerations, does not necessarily indicate fetal distress. Under normal circumstances in the near-term fetus, low variability will change to a high variability with periodic accelerations. In the term human newborn infant

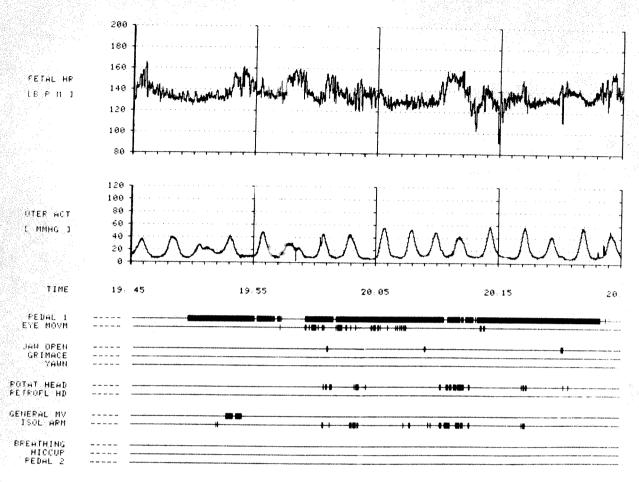


Fig. 3. Recording state 2F (active sleep) (No. 2).

and in the term human fetus, episodes of quiet sleep do not exceed 40 minutes.^{3, 7} Lack of this change for more than 40 minutes should be considered as a possible sign of fetal distress, particularly when beat-tobeat heart rate variability is diminished or even absent.

Richardson et al.8 observed fetal breathing activity and gross fetal body movements during the first stage of labor in 20 healthy term pregnancies. The real-time ultrasound transducer was directed at the fetal trunk. They found that patterns of increased fetal breathing activity were accompanied by gross fetal body movements for periods lasting 20 to 60 minutes of every 1:0 to 1.5 hours of the time observed. Heart rate variability was usually increased during the periods when letal breathing and other activity were present, which is comparable to state 2F. Periods with absence of fetal breathing and other movements were also accompanied by a low fetal heart rate variability, which is comparable to state 1F. The intermittent patterns of increased body movements and heart rate variability continued throughout the first stage of labor.

These observations by Richardson et al. compare well with our results. Apparently they also have been observing periodic behavioral states 2F and 1F.

We observed that during periods of state 1F the uterine contractions have virtually no effect on the fetal heart rate pattern. This is not surprising given the fact that it is very difficult to wake up a newborn infant when in state 1 (quiet sleep). Visser et al. studied the effect of external stimulation in near-term pregnancies. In each of the fetuses two episodes of low heart rate variability similar to state 1F were examined. Stimulation was applied during one period of low fetal heart rate variability by "shaking the fetus through the maternal abdomen." No stimulation was given during a subsequent period of low fetal heart rate variability. No differences between the groups were seen in the duration of the low fetal heart rate variability or the incidence of breathing or body movements.

Decreased heart rate variability has been regarded as a sign of fetal distress.¹⁰ In our study, we have observed that in the normal, healthy term fetus, periods of low heart rate variability correspond to the presence of behavioral state 1F (quiet sleep). Therefore periods of low heart rate variability do not necessarily indicate fetal distress. Diminished fetal oxygenation should, however, be considered when episodes of low heart rate variability exceed 40 minutes, particularly when the

short-term variability in fetal heart rate is also decreased and/or late decelerations are present.

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The first-trimester ultrasonographic diagnosis of conjoined twins

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The prenatal ultrasonographic diagnosis of conjoined twins in the first trimester is described. The ultrasonographic criteria are discussed together with implications for management. (AM J OBSTET GYNECOL 1985;152:833-5.)

Key words: Conjoined twins, ultrasound, prenatal diagnosis

Conjoined twinning is a rare anomaly estimated to occur in approximately 1% of monozygotic twins. The use of real-time ultrasound in the prenatal diagnosis of conjoined twins has been described by several authors. The case described here is to our knowledge the earliest ultrasonographic documentation of this rare entity.

Case report

A 35-year-old woman, para 3-0-0-3, was referred to The Johns Hopkins Hospital, Department of Radiology, at 8.5 weeks' postmenstrual age for assessment of gestational age. An Aloka 250 3.5 MHz linear array transducer was used to obtain a crown-rump length of 2.4 cm (consistent with 9.1 weeks' postmenstrual age). There was a single amniotic sac with normal amniotic

fluid. Imaging of the fetal pole showed a bifid appearance with a single heartbeat present (Fig. 1). Because of the abnormal appearance of the fetal pole, the possibility of conjoined twins was raised and repeat ultrasonography was scheduled in 4 weeks to confirm the diagnosis.

A second sonogram obtained with the Aloka 256 5.0 MHz transducer at 13 weeks' postmenstrual age showed a twin gestation with biparietal diameters of 2.6 cm (consistent with 13.7 weeks), a single amniotic sac, and normal amniotic fluid (Fig. 2). The fetuses were active yet maintained a constant orientation to each other and appeared to be joined at the thorax with a single heart visible.

The patient was counseled regarding the poor prognosis for conjoined twins with a single heart. She elected termination, but prior to termination she presented to the labor and delivery suite with bleeding and cervical dilatation to 2 cm. Prostaglandin E₂ suppositories were used to evacuate the pregnancy. Thoracopagus conjoined twins were delivered with a weight of 100 gm (Fig. 3).

Autopsy of the fetuses confirmed the diagnosis of conjoined twins joined at the thorax with a single heart

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Fig. 1. Ultrasonogram of conjoined twins at 8.5 weeks' postmenstrual age. *Arrows* indicate fetal heads.

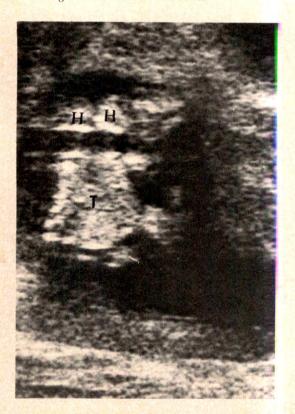


Fig. 2. Ultrasonogram of conjoined twins at 13 weeks' postmenstrual age, showing heads (*H*) and thorax (*T*).



Fig. 3. Postmortem photograph of conjoined twins.

and two aortas. The fetal lungs were complete and separate in each twin. The gastrointestinal tracts were fused at the distal duodenum for a length of 2 mm, and a single pancreas with two dorsal lobes and one ventral lobe was present. The urinary tract of one fetus was complete, but in the second twin a single kidney was present.

Comment

The sonographic and radiologic findings associated with conjoined twins have been summarized by Koontz et al.² These include (1) the lack of a separating membrane, (2) inability to separate the fetal bodies, (3) detection of other anomalies in a twin gestation, (4) more than three vessels present in the umbilical cord, (5) both fetal heads persistently at the same level, (6) backward flexion of the cervical spine, (7) a narrow space between the lower cervical and upper thoracic spine, and (8) no change in the relative positions of the fetuses despite attempts at manual manipulation of the twins.²

This case illustrates the possibility of the first-trimester diagnosis of thoracopagus conjoined twins. The bifid appearance of the first-trimester fetal pole should be added to the list of sonographic features of conjoined twins.

There is a higher incidence of early malformations in conjoined twins as in monozygotic twins in general. Smith¹ cited a 10% to 20% incidence of early defects in conjoined twins unrelated to the point of juncture. As in separate monozygotic twins, these mal-

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formations may not be concordant. Ultrasound is important, therefore, not only in the early diagnosis of this entity but also in evaluating the fetuses for other anomalies that may affect outcome. This information is essential in predicting the possibility of successful separation and in counseling the parents regarding prognosis.

The possibility of conjoined twins must be considered when the diagnosis of monoamniotic twins is made. The

first-trimester diagnosis of this rare anomaly is ideal if termination is to be offered as an option in those cases with poor prognosis.

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Long-term risk of menstrual disturbances after tubal sterilization

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We analyzed data from the Walnut Creek Contraceptive Drug Study to compare the menstrual characteristics of 719 women who had tubal sterilizations and 1083 women whose partners had undergone a vasectomy. Study participants were enrolled from 1968 to 1972 and followed up through 1976. The tubal sterilization group had slightly increased, though in most instances not statistically significant, risks of moderate to severe menstrual cramps and adverse menstrual bleeding. At follow-up intervals longer than 2 years, the tubal sterilization group had significantly increased risks of abnormal menstrual cycles and combinations of two or more adverse menstrual outcomes. Tubal sterilization was not associated with an increased risk of premenstrual symptoms. These findings suggest that the types of tubal sterilization procedures which were performed during the early 1970s possibly carry some increased risk of menstrual disturbances, particularly abnormal cycles, and that it may take more than 2 years for the increased risk to become apparent. (AM J OBSTET GYNECOL 1985;152:835-41.)

Key words: Menstrual disturbances, menstruation, tubal sterilization, female sterilization, premenstrual symptoms

Despite more than 30 years of study, whether tubal sterilization increases the risk of menstrual disturbances is still unresolved. Two recent large, prospective studies have found little evidence of an increased prevalence of menstrual disorders after tubal sterilization. Neither study, however, included a control group of nonsterilized women and follow-up was limited to 1 or 2 years. Although both studies' findings are somewhat reassuring, there is concern that it may take

several years for menstrual disturbances to develop after tubal sterilization. Furthermore, since both studies lacked a control group, they were unable to evaluate whether menstrual disturbances are more prevalent after sterilization operations than would be expected among nonsterilized women after a comparable period of time.

Two other issues also remained unresolved. One concerns the existence of a "post-tubal ligation syndrome." This syndrome has never been specifically defined but may consist of a variety of menstrual disorders including irregular cycles, dysmenorrhea, increased menstrual bleeding, and intermenstrual bleeding. 4-6 The two recent prospective studies examined menstrual symptoms on an individual basis but did not evaluate combinations of symptoms or "menstrual syndromes." A second concern is the possibility that women undergoing tubal sterilization may develop premenstrual symptoms or have preexisting premenstrual complaints exacerbated. This possibility has not been further

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Reprint requests: Dr. Frank DeStefano, Center for Environmental Health, Centers for Disease Control, Atlanta, GA 30333. evaluated since an initial report by Hargrove and Abraham.⁵

We analyzed data from the Walnut Creek Contraceptive Drug Study to try to answer some of the remaining questions about the association between tubal sterilization and menstrual disturbances. The Contraceptive Drug Study prospectively gathered information on menstrual characteristics from study participants and included women who underwent tubal sterilization and women whose partners had a vasectomy. Following information was available for longer than 4 years on a number of women. In addition to individual menstrual symptoms, we also evaluated premenstrual symptoms and combinations of menstrual disorders.

Methods

The Contraceptive Drug Study. The Contraceptive Drug Study was a long-term, prospective study designed to evaluate the noncontraceptive effects of gral contraceptives. The methods of the Contraceptive Drug Study have previously been reported in detail.7 Briefly, members of the Kaiser-Permanente Family Health Plan of Walnut Creek, California, were enrolled in the study from December, 1968, through February, 1972. Participants were recruited from women who were seeking routine medical or gynecologic checkups. At the initial enrollment, all women underwent a special automated multiphasic laboratory examination which included detailed questionnaires on past medical, surgical, reproductive, and contraceptive use history. From 1971 to 1976, study participants periodically underwent repeat automated multiphasic laboratory examinations.

Study groups. Of the 17,939 women enrolled in the study, 13,749 had at least one follow-up visit. Since *7% of the study participants were white, we restricted the analysis to white women. Among the white women with at least one follow-up visit, we identified 719 women who underwent a tubal sterilization procedure at some time between enrollment into the study and the last follow-up visit (tubal sterilization group). During the follow-up interval, if a woman began using a surgical method of birth control but provided no history of a tubal sterilization, hysterectomy, or bilateral oophorectomy, we assumed that her partner had undergone a vasectomy; there were 1083 such women (vasectomy group). The date of sterilization operation (either tubal sterilization or vasectomy) was taken to be the date a participant reported beginning a surgical method of birth control.

Study outcomes. At each automated multiphasic laboratory examination, study participants completed a self-administered questionnaire which contained a series of questions on the usual nature of their menstrual characteristics and premenstrual symptoms during the

previous year. Based on the information on the questionnaires, we defined three specific menstrual disorders: (1) abnormal cycles—cycles that were reported as not usually regular and/or not of 24 to 35 days' length; (2) adverse bleeding—two or more of (a) menstrual flow duration ≥ 6 days, (b) average use of ≥ 12 full pads or tampons per period, (c) moderate or severe large clots with menstrual flow, and (d) moderate or severe spotting or intermenstrual bleeding; (3) moderate or severe menstrual cramps.

Since a "post-tubal ligation syndrome" has not been defined, we arbitrarily defined a combination of symptoms which for purposes of reference we shall call the "adverse menstrual symptom complex." The adverse menstrual symptom complex was defined as consisting of at least two of the above-defined menstrual disorders. As a further measure of severity of menstrual symptoms, we also evaluated self-reported physician visits because of menstrual problems.

We used Hargrove and Abraham's⁵ classification as a guide for grouping the premenstrual symptoms into three categories: (1) moderate or severe irritability and/or moodiness, (2) moderate or severe swelling and/or weight gain, and (3) moderate or severe headache and/or dizziness.

Analysis. The analysis focused on comparing the various menstrual and premenstrual outcomes in the tubal sterilization group to the vasectomy group at three different follow-up intervals: 6 to 24 months, 25 to 48 months, and 49 to 87 months after the sterilization operation date. The presterilization visit was taken to be the closest visit prior to the sterilization date. The mean interval from the presterilization visit to the operation date was 15 months for the vasectomy group and 16 months for the tubal sterilization group. Since the presterilization visit could have occurred at various times prior to the sterilization date, the intervals from the sterilization date to the follow-up date do not necessarily correspond to the intervals from the presterilization visit to the follow-up date.

We used a logistic regression model to estimate the odds ratio, the 95% confidence interval of the odds ratio, and the predicted proportion of the study outcomes.⁸ In addition to group (tubal sterilization or vasectomy) the model included, and simultaneously adjusted for, several variables that could influence menstrual status: presterilization status of the menstrual or premenstrual variable being evaluated, presterilization age, contraception, history of gynecologic disorders, gravidity, body mass index (gm/cm²), cigarette smoking, education, and religion. In addition to the preceding presterilization characteristics, the model also adjusted for differences in number of follow-up visits and total follow-up interval (that is, the interval from the presterilization visit to the follow-up visit). We tested for

interactions between group and presterilization status of the menstrual or premenstrual variables by the likelihood ratio test. In the results, if the odds ratio and 95% confidence interval are the same for the different presterilization status groups, then the interaction is not significant. If the odds ratio and 95% confidence interval differ according to presterilization status, then the interaction is significant and indicates that the effect of tubal sterilization is different for women with abnormal function before sterilization than for women with normal presterilization function.

Results

Group characteristics. Compared to the vasectomy group, the tubal sterilization group was younger, was more likely to have been pregnant or post partum at the presterilization visit, and included slightly more cigarette smokers, more Catholics, and fewer Protestants (Table I). The distribution of gravidity, history of gynecologic disorders, and body mass index was similar in the two groups. All the preceding variables were included in the logistic model which adjusted for any differences between the groups on these characteristics.

Menstrual cycle. The findings on menstrual cycle varied depending on whether women had normal or abnormal cycles at the presterilization visit (Table II). Among women who had normal presterilization cycles, the prevalence of abnormal cycles was little different in the two groups 6 to 24 and 25 to 48 months after the sterilization date. At 49 to 87 months after the sterilization date, however, the tubal sterilization group had a significantly increased prevalence of abnormal cycles; abnormal cycles were nearly three times more prevalent in the tubal sterilization group than in the vasectomy group.

Among women with abnormal presterilization cycles, one of the most striking findings was that the proportion with abnormal cycles at the follow-up visits varied from 0.12 to 0.51, indicating that in both groups there was a strong tendency for abnormal cycles to become normal. There was little difference between the two groups at 6 to 24 months. However, at 25 to 48 months after the sterilization date, the tubal sterilization group had a significantly increased prevalence of abnormal cycles. Too few women with abnormal presterilization cycles were followed up beyond 48 months to be able to evaluate the association between group and abnormal cycles at this longest follow-up interval.

Menstrual cramps. Except for the 6- to 24-month follow-up interval, tubal sterilization had a similar influence on menstrual cramps among women with none to mild cramps before sterilization as among women with moderate to severe cramps before sterilization (Table III). At the 6- to 24-month follow-up interval, the tubal sterilization group had a significantly in-

Table I. Selected presterilization characteristics by group, the Walnut Creek Contraceptive Drug Study, 1969 to 1976

	Percen characte	
Characteristic	Vasectomy (N = 1083)	$Tubal \\ sterilization \\ (N = 719)$
Age (yr)		
18-29	33.2	49.9
30-39	47.5	41.9
40-51	19.3	8.2
Contraception		
Pregnant or postpartum	5.6	22.2
Oral contraceptives	45.3	35.2
Other or none	49.1	42.6
Gravidity		
0 '	1.6	2.5
1-2	47.9	45.8
≥3	50.0	51.0
Education		- 4,00
Less than 12 years	6.3	7.0
High school graduate	36.5	42.1
Some college or technical school	34.8	32.9
College graduate	21.9	17.4
Religion		
Protestant	57.6	50.1
Catholic	25.6	31.0
Other or none	16.2	18.2
Current cigarette smoker	30.7	34.6
History of gynecologic disorders†	15.2	15.2
Body mass index		
1.00-1.89	4.9	6.0
1.90-2.39	64.1	65.3
2.40-2.99	26.4	25.2
3.00-7.00	4.6	3.5

^{*}Distributions that do not add up to 100% are due to exclusion of unknowns

creased prevalence of moderate to severe cramps among women who had no or mild cramps before sterilization. In contrast, among women who had had moderate to severe cramps before sterilization there was a lower prevalence of moderate to severe cramps in the tubal sterilization group compared to the vasectomy group, but this could have been a chance finding. At 25 to 48 and 49 to 87 months after the sterilization date, the tubal sterilization group had modestly increased risks of moderate to severe cramps which could have been due to chance.

Adverse bleeding. At all follow-up intervals the association between tubal sterilization and adverse bleeding was similar for women who had adverse bleeding before sterilization and for women who did not have adverse bleeding before sterilization (Table IV). The tubal sterilization group had a moderately increased risk of adverse bleeding at all follow-up intervals, but in no instance was the increase statistically significant.

Adverse menstrual symptom complex. The risk of

[†]Pelvic inflammatory disease, uterine infection, endometriosis, uterine fibroids, ovarian cvsts.

Table II. Prevalence of abnormal menstrual cycles* at selected intervals after sterilization, by group and status before sterilization, the Walnut Creek Contraceptive Drug Study, 1968 to 1976

		T	T		· · · · · · · · · · · · · · · · · · ·				
Months after sterilization Group	n	Prevalence of abnormal cycles at follow-up†							
		Among women who had normal cycles before sterilization			Among women who had abnormal cycles before sterilization				
		Proportion	Odds ratio	95% Confidence interval	Proportion	Odds ratio	95% Confidence interval		
6-24	Vasectomy	687	80.0	1.0	Referent	0.25	1.0	Referent	
	Tubal sterilization	434	0.10	1.22	0.80 - 1.86	0.29	1.22	0.80-1.86	
25-48	Vasectomy	441	11.0	1.0	Referent	0.12	1.0	Referent	
	Tubal sterilization	291	0.12	1.10	0.65 - 1.86	0.51	7.64	1.74-33.4	
49-87	Vasectomy	214	0.94	1.0	Referent	Ins	ufficient d	lata	
	Tubal sterilization	143	0.11	2.77	1.09-7.05				

^{*}Abnormal cycles were defined as not usually regular and/or not of 24 to 35 days' length.

Table III. Prevalence of moderate to severe mensurual cramps at selected intervals after sterilization, by group and status before sterilization, the Walnut €meek Contraceptive Drug Study, 1968 to 1976

			Prevalence of moderate to severe cramps at follow-up*							
			Among women who had no or mild cramps before sterilization			Among women who had moderate or severe cramps before sterilization				
Months after sterilization	ter	n	Propoziwn	Odds ratio	95% Confidence interval	Proportion	Odds ratio	95% Confidence interval		
6-24	Vasectomy	682	0.12	1.0	Referent	0.54	1.0	Referent		
	Tubal sterilization	425	0.18	1.55	1.06 - 2.28	0.44	0.66	0.37 - 1.19		
25-48	Vasectomy	439	0.1%	1.0	Referent	0.50	1.0	Referent		
	Tubal sterilization	286	0.2	1.37	0.92 - 2.02	0.57	1.37	0.92-2.02		
49-87	Vasectomy	208	0.19	1.0	Referent	0.57	1.0	Referent		
	Tubal sterilization	139	0.2^{ω}	1.19	0.64 - 2.22	0.62	1.19	0.64-2.22		

^{*}Both the proportion and odds ratio with 95% confidence interval were calculated by means of a logistic regression model that adjusted for age, previous contraceptive practices, gravidity, history of gynecologic disorders, body mass index, cigarette smoking, education, and religion at the presterilization visit, as well as number of follow-up visits and total follow-up interval (from presterilization visit to follow-up visit).

an adverse menstrual symptom complex for the tubal sterilization group relative to the vasectomy group at follow-up intervals from 6 to 48 months was similar for women with and without an adverse menstrual symptom complex at the presterilization visit (Table V). At the 6- to 24-month follow-up interval, the tubal sterilization group had a slightly, but not significantly, increased risk of an adverse menstrual symptom complex. At the 25- to 48-month follow-up interval, however, the tubal sterilization group had a significantly increased risk of an adverse menstrual symptom complex (odds ratio = 1.69). At the 49- to 87-month followup interval, among women who did not have an adverse menstrual symptom complex at the presterilization visit, the tubal sterilization group had a slightly, although not significantly, increased risk of an adverse

menstrual symptom complex. Among women who had an adverse menstrual symptom complex at the presterilization visit, however, the tubal sterilization group was at greatly increased risk for persistence of an adverse menstrual symptom complex and the increased risk was not likely to be explained by chance.

Although the adverse menstrual symptom complex results suggest that the tubal sterilization group may have had more menstrual disturbances, there was no significant difference between the two groups in self-reported physician visits for menstrual problems. The odds ratios for physician visits for menstrual problems were 0.70, 0.92, and 0.96 at the three follow-up intervals and none was significantly different from 1.0.

Premenstrual symptoms. The three premenstrual symptom categories showed little association with tubal

[†]Both the proportion and odds ratio with 95% confidence interval were calculated by means of a logistic regression model that adjusted for age, contraceptive practices, gravidity, history of gynecologic disorders, body mass index, cigarette smoking, education, and religion at the presterilization visit, as well as number of follow-up visits and total follow-up interval (from presterilization visit to follow-up visit).

Table IV. Prevalence of adverse bleeding* at selected intervals after sterilization, by group and status before sterilization, the Walnut Creek Contraceptive Drug Study, 1968 to 1976

			Prevalence of adverse bleeding at follow-up**						
			Among women who did not have adverse bleeding before sterilization			Among women who had advers bleeding before sterilization			
Months after sterilization	Group	n	Proportion	Odds ratio	95% Confidence interval	Proportion	Odds ratio	95% Confidence interval	
6-24	Vasectomy	683	0.12	1.0	Referent	0.45	1.0	Referent	
	Tubal sterilization	425	0.15	1.34	0.96 - 1.88	0.52	1.34	0.96-1.88	
25-48	Vasectomy	439	0.16	1.0	Referent	0.51	1.0	Referent	
	Tubal sterilization	286	0.18	1.22	0.81 - 1.82	0.56	1.22	0.81 - 1.82	
49-87	Vasectomy	208	0.15	1.0	Referent	0.55	1.0	Referent	
	Tubal sterilization	140	0.22	1.58	0.85 - 2.92	0.66	1.58	0.85 - 2.92	

^{*}Adverse bleeding defined as two or more of the following: (1) menstrual bleeding ≥6 day's duration, (2) average of 12 or more full pads or tampons per period, (3) moderate or severe large clots with menstrual flow, and (4) moderate or severe spotting or bleeding between periods.

sterilization status. For all three categories the findings were similar regardless of whether women had the premenstrual symptoms before sterilization. At the three follow-up intervals the odds ratios for moderate or severe irritability and/or moodiness were 0.87, 0.67, and 0.81; the only one that was statistically significant was the odds ratio of 0.67 at the 25- to 48-month followup interval (95% confidence interval = 0.45 to 0.98). The corresponding odds ratios for moderate or severe headache or dizziness were 0.91, 0.82, and 0.84, and for moderate or severe swelling or weight gain they were 1.35, 0.72, and 1.03; none was significantly different from 1.0.

Comment

This is the only study of which we are aware that has prospectively gathered information on specific menstrual characteristics for longer than 2 years after tubal sterilization. Our findings lend some support to the concern that it may take several years after tubal sterilization for an increased risk of menstrual disturbances to become apparent. Two or more years after tubal sterilization there was an increased risk of menstrual cycles that were irregular and/or not of 25 to 34 days' duration. Women who had tubal sterilizations were also at somewhat increased risk of having a combination of at least two adverse menstrual characteristics. Moderate to severe cramps and adverse bleeding were both more prevalent among sterilized women at almost all followup intervals, but these increased risks could likely have been chance findings. Our results do not support the hypothesis that tubal sterilization may increase the risk of premenstrual symptoms.

For both sterilized and nonsterilized women, the occurrence of menstrual changes was dependent on presterilization menstrual status. For all menstrual variables, relatively few women in both groups who were normal before sterilization developed abnormal function after sterilization, whereas a large proportion of women who were abnormal before sterilization changed to normal at the follow-up visits. The effects of tubal sterilization also varied depending on presterilization menstrual status. For both menstrual cycle and combinations of adverse menstrual characteristics it was among women who were abnormal prior to sterilization that tubal sterilization carried the greatest risk of abnormal function at the long-term follow-up visits (that is, sterilized women were less likely to change to normal).

If tubal sterilization leads to menstrual disturbances, how it may do so is uncertain. It has been suggested that such changes may be mediated by disruption of the uteroovarian blood supply with resultant disturbances of ovulatory function.6 Regardless of the mechanism, a disturbance in ovulatory function might result in the menstrual cycle changes we observed. Disorders of ovulation can result in short cycle lengths in the case of luteal phase defects and in long cycle lengths in the case of oligoovulation. Our data do not allow determination of direction of cycle length abnormalities, but nonetheless our results are consistent with the possibility suggested by a few laboratory studies5.6,9 that tubal sterilization may affect subsequent ovulatory function in some women.

Since the Contraceptive Drug Study was principally a study of side effects of oral contraceptives and was

[†]Both the proportion and odds ratio with 95% confidence interval were calculated by means of a logistic regression model that adjusted for age, contraceptive practices, gravidity, history of gynecologic disorders, body mass index, cigarette smoking, education, and religion at the presterilization visit, as well as number of follow-up visits and total follow-up interval (from presterilization visit to follow-up visit).

Table V. Prevalence of adverse menstrual symptom complex* at selected intervals after sterilization, by group and status before sterilization, the Walnut Creek Contraceptive Drug Study, 1968 to 1976

			Prevalence of adverse menstrual symptom complex						
			Ameng women who did not have adwerse menstrual symptom complex before sterilization		Among women who had adv menstrual symptom complex b sterilization		plex before		
Months after sterilization	Group	n	Proportion	Odds ratio	95% Confidence interval	Proportion	Odds ratio	95% Confidence interval	
6-24	Vasectomy	687	0.09	1.0	Referent	0.28	1.0	Referent	
	Tubal sterilization	434	0.12	1.26	0.82 - 1.91	0.33	1.26	0.82-1.91	
25-48	Vasectomy	442	0.09	1.0	Referent	0.42	1.0	Referent	
	Tubal sterilization	291	0.14	1.69	1.06 - 2.70	0.54	1.69	1.06-2.70	
49-87	Vasectomy	212	0.97	1.0	Referent	0.12	1.0	Referent	
	Tubal sterilization	143	0.98	1.25	0.55 - 2.85	0.83	36.4	1.33-997	

^{*}The adverse menstrual symptom complex consists of two or more of the following: (1) abnormal cycle, (2) adverse menstrual bleeding, and (3) moderate or severe menstrual cramps.

not designed to evaluate poststerilization menstrual changes, there are limitations of the data for the current analysis. The method we used to infer that a woman's partner had had a vasectomy may have led to some inaccuracies in our risk estimates. Since the Contraceptive Drug Study questionnaires did not specifically ask about partner's vasectomy status, we assumed the partner had a vasectomy if the participant stated she was using a surgical method of birth control but provided no history of tubal sterilization, hysterectomy, or bdateral oophorectomy. This may have resulted in some misclassification if some women who had tubal sterilizations failed to report the sterilization but did report that they were using a surgical method of birth control This misclassification would have tended to drive the odds ratio estimate toward 1.0 and thus would have obscured any association between tubal sterilization and adverse menstrual outcomes. Information from a brief questionnaire administered to study participants in 1977, however, indicates that misclassification was probably small. The 1977 questionnaire specifically asked study participants if their husbands had had wasectomies and the year of vasectomy. Eighty-five percent of the vasectomy group responded to the 1977 questionnaire and the agreement between the inferred year of vasectomy from the automated multiphasic laboratory information and the vasectomy year reported on the 1977 questionnaire was within 1 year for 79% and within 2 years for 92%. In comparison, 93% of the tubal sterilization group responded to the 1977 questionnaire and the agreement between the automated multiphasic laboratory tubal sterilization year and the year of tubal sterilization provided on the 1977 questionnaire was within 1 year for 93% and within 2 years for 97%.

To infer that a woman's partner had had a vasectomy, we had to exclude women who reported having had a hysterectomy. (Eight percent of Contraceptive Drug Study participants with an intact uterus at enrollment had a hysterectomy by their last follow-up visit.) If, as has been suggested by some studies,10,11 women who underwent tubal sterilization had an increased risk of hysterectomy, excluding women who had hysterectomy from the analysis may have resulted in underestimates of the risks of menstrual disturbances after tubal sterilization. However, a prospective study in the United Kingdom has not found an increased rate of hysterectomy among women who had tubal sterilizations compared with women whose husbands had vasectomies. 12 Given the equivocal evidence on the association between tubal sterilization and subsequent hysterectomy, we do not believe that excluding women who had hysterectomy had a large influence on our results.

Reporting bias is another potential problem. If any participants in the Contraceptive Drug Study were aware of the possibility that tubal sterilization may cause menstrual problems, there may have been some biased overreporting of menstrual problems by the tubal sterilization group. For several reasons we do not think this is likely to have occurred. First, the study was principally designed to evaluate oral contraceptive side effects; the association between tubal sterilization and menstrual disturbances was not considered in the design and conduct of the study, reducing the likelihood that design of the questionnaire had an important influence on participant response. Second, women in both the tubal sterilization and vasectomy groups served as controls in the study, and thus they were probably treated similarly. Finally, if the observed increased risks were due simply to reporting bias, we

[†]Both the proportion and odds ratio with 95% confidence interval were calculated by means of a logistic regression model that adjusted for age, contraceptive practices, gravidity, history of gynecologic disorders, body mass index, cigarette smoking, education, and religion at the presterilization visit, as well as number of follow-up visits and total follow-up interval (from presterilization visit to follow-up visit).

would have expected to see some indication of increased reporting of premenstrual symptoms among the tubal sterilization group as well. That the prevalence of premenstrual symptoms in the tubal sterilization group was similar to or less than that in the vasectomy group suggests that the tubal sterilization group probably was not overreporting menstrual symptoms.

We were not able to determine the timing of the sterilization procedure (that is, pregnancy-associated or interval) or the method of tubal occlusion. However, since most (93%) of the procedures were done before 1975, probably half or more were pregnancy-associated and nearly all were probably done by partial salpingectomy or by unipolar electrocoagulation. 18, 14 Little is known about the influence of the timing of the sterilization procedure on subsequent menstruation, but there is evidence that different methods of tubal occlusion may have different effects on menstruation. Neil et al. 15 have reported the only other study of menstrual symptoms that included wives of men who had vasectomy as controls. Their retrospective study found that menstrual blood loss and menstrual pain were both higher in women who underwent laparoscopic sterilization (most likely by unipolar coagulation) than in women who had had tubal ligation performed by a modified Pomeroy (partial salpingectomy) technique. In addition, preliminary findings from the Centers for Disease Control study² and from studies of hormonal changes9 and endometriosis16 after tubal sterilization suggest that procedures that cause a relatively large degree of tissue destruction, such as unipolar coagulation and partial salpingectomy, may be associated with a higher risk of menstrual problems than are methods of occlusion that cause relatively little tissue destruction, such as spring clips and, perhaps, silicone rubber bands and bipolar coagulation. Probably very few, if any, of the women in the Contraceptive Drug Study underwent tubal occlusion by clips, bands, or bipolar coagulation and our findings may not pertain to these methods of occlusion.

In conclusion, our findings suggest that the tubal sterilization procedures that were performed in the early 1970s possibly carry some increased risk of menstrual disturbances, particularly abnormal cycles, and that it may take more than 2 years for the increased risk to become apparent. How applicable these findings are to the newer methods of tubal occlusion used more commonly today is not clear. We are currently conducting a long-term prospective study of specific methods of tubal sterilization to determine the influence of timing and degree of tissue destruction on subsequent menstruation.

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Malignant ovarian germ cell tumors: A review of thirty-six cases

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Thirty-six patients with malignant ovarian germ cell tumers were treated between 1972 and 1983, including 16 with immature teratoma, five with endodermal sinus tumor, seven with dysgerminoma, and eight with mixed germ cell tumors. The median age at presentation was 18 years and mean primary tumor diameter was 18 cm. Twenty-five of the 27 patients who were treated with multiple-agent chemotherapy underwent second-look procedures, only two of which revealed persistent malignancy. No patients have developed recurrence after a negative second-look operation. Two of the three patients with failure of initial chemotherapeutic regimens had complete remissions with second regimens. Two patients have died of malignancy, one who presented with a Stage IA mixed germ cell tumor and one noncompliant patient with a Stage IA, grade 2 immature teratoma. The other 34 patients are alive without evidence of disease from 21 to 141 months, with a median follow-up of 68 months. These data confirm that multiple-agent chemotherapy has dramatically improved the prognosis for patients with malignant nondysgerminomatous ovarian germ cell tumors. (AM J OBSTET GYNECOL 1985; #52:842-6.)

Key words: Ovarian germ cell, chemotherapy, tumors

Dramatic progress has been made in the past 10 wears in the treatment of ovarian germ cell malignancies. Prior to the introduction of multiple-agent chemotherapy the cure rates for nondysgerminomatous ovarian germ cell tumors were poor. Approximately 10% of patients with endodermal sinus tumors survived prior to 1970.1.2 Mixed germ cell tumors had a simularly poor prognosis with only eight survivors among 214 patients reported on in a 1976 review of the world's literature.3 Immature teratomas also had a signiacant mortality rate in the prechemotherapy era. The 1976 review of Norris et al.4 of the 58 cases of ovarian mmature teratomas in the Armed Forces Institute of Pathology registry revealed survivals of 81%, 60%, and 30%, respectively, for patients with grade 1, 2, and 3 tumors. Only one of 10 patients with grade 1 to 3 metastases survived.4 Of all the germ cell malignancies only pure dysgerminomas had a high cure rate prior to 1970. This was due to the exquisite radiosensitivity of these tumors. In dysgerminoma, although patient survival rates as low as 27% have been reported, 5.6 most series report 5-year survival rates of 70% to 92%.

Multiple-agent chemotherapy has dramatically improved the prognosis of patients with malignant evar-

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Table I. Staging at diagnosis

	Stage					
Tumor	IA (No.)	IC (No.)	HC (No.)	III (No.)		
Immature teratoma	13	1	1	1		
Endodermal sinus tumor	3	1		1		
Dysgerminoma	4		1	2		
Mixed germ cell tumor	6	1		1		

ian germ cell tumors. Rutledge, in 1968, first reported three complete responses in 22 patients with malignant teratomas treated with intravenous melphalan. In 1975, Smith and Rutledge reported a much higher response rate with a vincristine—actinomycin D—Cytoxan (VAC) regimen.

Effective second-line chemotherapy for malignant ovarian germ cell tumors has also been developed. Slayton et al. ¹⁰ reported, in 1977, on two patients who had failure of VAC and then responded to treatment with vinblastine-bleomycin-cisplatin (VBP). The successes of the VBP regimen in the treatment of testicular germ cell tumors led to use of this regimen in ovarian germ cell malignancies both as primary treatment and for VAC failures. ¹¹⁻¹³

The purpose of this study is to report the experience at the University of California (Irvine) Medical Center, Division of Gynecologic Oncology, with a group of 36 patients with malignant ovarian germ cell tumors treated between 1972 and December, 1983, and fol-

Table II. Summary of cases and chemotherapy given

Tumor	No. of patients	Chemotherapy	Status and months since diagnosis
Immature teratoma	2	VAC × 6	Died of disease, 21 mo. NED 120 mo
	. 1	$VAC \times 7$	NED 122 mo
	4	$VAC \times 9$	NED 30,83,38,50 mo
	Ĩ	$VAC \times 10$	NED 83 mo
	1	$VAC \times 12$	NED 23 mo
	î	$VAC \times 13$	NED 141 mo
	. 1	$VAC \times 21$	NED 89 mo
	. 1	$VAC \times 1$.	NED 34 mo
	-	VinCyt. \times 3; then VBP \times 4	
	. 1	VBP/Act. D-Cyt./	NED 26 mo
	*	Adria. × 2;	
		then VAC × 12	
Endodermal sinus tumor	2	$VAC \times 9$	NED 39,52 mo
Engogermai sinus tumoi	Ī	$VAC \times 12$	NED 102 mo
	i	VAC × 14	NED 87 mo
	1	Cyt5-FU-Act.	NED 154 mo
		D × 11	
Histologic features of mixed germ cell tumors			
Endodermal sinus tumor, dysger-	1	$VAC \times 6$	NED 40 mo
minoma			NED 15 mo
Dysgerminoma, endodermal sinus tumor, immature teratoma, embryonal, syncytiotrophoblast		VAC × 8	
Immature teratoma, embryonal	1	$VAC \times 9$	NED 26 mo
Immature teratoma, endodermal sinus tumor	2	VAC × 9	NED 48,14 mo
Dysgerminoma, endodermal sinus tumor	1	$VBP \times 4$	NED 12 mo
Dysgerminoma, embryonal, immature teratoma	1	Cyt5-FU-Act. D	NED 145 mo
Immature teratoma, endodermal sinus tumor, embryonal	ī	VAC × 3; VBP × 2; Ad- ria./VP-16, cisplatin, metho-	Died of disease, 19 mo
		trexate + CV rescue × 1	
Dysgerminoma	1	VAC × 10	Positive second look; then radiation; NED
			84 mo

NED = No evidence of disease; Vin. = vincristine; Cyt. = cytoxan; Act. D = actinomycin D; Adria. = Adriamycin; 5-FU = 5fluorouracil; CV = citrovorum factor.

lowed up to November, 1984, including 27 treated with multiple-agent chemotherapy.

Material and methods

The files of the Division of Gynecologic Oncology, Department of Obstetrics and Gynecology, at the University of California (Irvine) Medical Center were reviewed to locate all patients with malignant ovarian germ cell tumors managed from 1972 to December, 1983. Patients were classified as having pure immature teratoma, dvsgerminoma, endodermal sinus tumor, embryonal carcinoma, or mixed germ cell tumor. Clinical data were abstracted and follow-up information was obtained on all patients to the time of death or until January, 1984.

Results

The ages of the patients ranged from 9 to 39 years, with a median of 18 years. Sixteen patients had immature teratoma, five had endodermal sinus tumor, seven had dysgerminoma, and eight had mixed germ cell tumor. The mean primary tumor diameters were 15.7, 22, 21, and 16 cm, respectively. The mean primary tumor diameter for the entire group was 18 cm. Only one patient had a primary tumor <10 cm in diameter, and only one patient with a Stage III dysgerminoma had bilateral ovarian tumors at initial diagnosis. The presenting stage (International Federation of Gynaecology and Obstetrics) is shown in Table I. Three of the immature teratomas were Norris grade 1, four were grade 2, eight were grade 3, and one was not graded.

Table III. VAC and VBP regimens

Drug	Dose	Schedule	***************************************
VAC regimen Vincristine (maximum dose 2.0 mg) Actinomycin D (maximum dose 0.5 mg Cyclophosphamide Methylprednisolone (Antiemetic)	1.5 mg/m ² 350 µg/m ² 150 mg/m ² 125 mg	Intravenous, every other week Intravenous, daily for 5 days every 4 wk Intravenous, daily for 5 days every 4 wk Intravenous, daily for 5 days	1 2 2
VBP regimen Vinblastine Bleomycin (maximum dose 30 U) Cisplatin Total: 4 courses maximum	12 mg/m ² 20 U/m ² 20 mg/m ²	Intravenous, on day 1 every 3 wk Intravenous, weekly for 7 wk, then again once in 10th wk Intravenous, daily for 5 days every 3 wk	

The histologic features of the mixed germ cell turnors are shown in Table II. Syncytiotrophoblast was noted in one mixed germ cell tumor.

Initial surgical management consisted of unilateral salpingo-oophorectomy in 26 cases and total abdominal hysterectomy with bilateral salpingo-oophorectoms in 10 cases. The conservative surgical approach of unilateral adnexectomy was adopted in the later years.

Tumor markers. Tumor markers were measured in eight of the 16 patients with immature teratoma. Two patients were referred with progressive disease and had serum α-fetoprotein levels of 992 and 152 ng/ml. Follow-up α-fetoprotein assays during therapy were negative. Both patients later had negative second-look laparotomies and remain without evidence of disease at 36 and 28 months, respectively. Seven patients with immature teratoma had serum human chorionic gonadotropin (hCG) assays done and one had a serum carcinoembryonic antigen assay done. These were all negative.

Two of the six patients with endodermal sinus turnor had serum α-fetoprotein and hCG pretreatment assays done. These assays were elevated prior to therapy and promptly became negative with treatment. Two wither patients with endodermal sinus tumor did not have pretreatment α-fetoprotein assays done but did have negative assays during therapy.

Three patients with mixed germ cell tumor had elevated α -fetoprotein but undetectable hCG prior to treatment. A fourth patient had elevated α -fetoprotein and hCG prior to therapy. All four patients had negative follow-up assays during therapy and remain without evidence of disease. Three other patients had negative α -fetoprotein and hCG assays prior to and during therapy. There was a correlation between disease status and α -fetoprotein serum levels in the one patient with mixed germ cell tumor that progressed and who died of disease. hCG and α -fetoprotein were assayed in only two patients with dysgerminomas. These assays were negative.

Treatment. After operation, 27 patients received

treatment with multiple-agent chemotherapy (Table II). Nine patients did not receive chemotherapy, six patients with dysgerminoma and three patients with Stage IA immature teratoma. The VAC and VBP regimens used are summarized in Table III.

Outcome. Only two of the 36 patients have died. One patient had a Stage IA mixed germ cell tumor consisting of immature teratoma, endodermal sinus tumor, and embryonal carcinoma. She had failure of VAC, VBP, VP-16, cisplatin, and high-dose methotrexate with citrovorum rescue and died 19 months after initial diagnosis. The second death was in a patient with a Stage IA, grade 2 immature teratoma. She refused chemotherapy initially. Eight months after initial diagnosis she presented elsewhere with an acute abdomen. Laparotomy revealed carcinomatosis with immature teratoma. She was treated at a third institution with a VAC regimen. She received six VAC courses but at second-look laparotomy persistent immature teratoma was found. She refused further therapy and died 2 months later.

Of the 29 patients with immature teratoma, endodermal sinus tumor, or mixed germ cell tumor, 26 have had negative second-look laparotomies. One additional patient had a negative second-look laparoscopy, and two patients refused a second-look operation. No patients have had recurrence of tumor after a negative second-look procedure.

The only positive second-look laparotomy was in the noncompliant patient with immature teratoma who died with disease. Three other patients with immature teratoma underwent laparotomies to document progression of disease, received additional chemotherapy, and then had negative second-look laparotomies. One patient had progression on a regimen consisting of VBP for one cycle, then Cytoxan—actinomycin D for one cycle, then Adriamycin for one cycle, and then a repeat of these drugs. Massive progression was documented at laparotomy. After 12 courses of VAC, she had a negative second-look laparotomy 26 months ago. A second patient received one course of VAC, followed by three courses of vincristine and Cytoxan when progression

was noted and documented at laparotomy. After four courses of VBP, she had a negative second-look laparotomy 34 months ago. A third patient initially had a 20 cm Stage IA, grade 1 immature teratoma. She was observed and developed a recurrence 6 months later. Total abdominal hysterectomy, bilateral salpingo-oophorectomy, and removal of a metastatic cul-de-sac nodule were done. She received VAC for six cycles and then had a negative second-look laparotomy; she was without evidence of disease when last contacted 120 months after initial diagnosis.

Seven of the 13 patients with immature teratoma who had negative second-look laparotomies were found to have grade 0 (mature teratoma) implants at the time of that operation. Mature teratoma implants were also noted in one of the 12 patients with endodermal sinus tumor or mixed germ cell tumor at the time of secondlook laparotomy. None of these eight patients with mature teratoma implants have had any clinical problems due to the implants. Two of these patients have undergone reexploration for pelvic masses found to be mature teratomas.

Only one patient with dysgerminoma was treated with chemotherapy. She presented with Stage III disease and was treated with 10 courses of VAC after operation. She was found to have persistent dysgerminoma in the pelvis at second-look laparotomy. She was then treated with whole-abdomen radiation therapy, 3000 rad to the upper abdomen and 4000 rad to the pelvis. She remains without evidence of disease 84 months since diagnosis. Another patient with a Stage IA dysgerminoma was observed after a unilateral salpingo-oophorectomy. She developed recurrence in the supraclavicular, mediastinal, and para-aortic regions 22 months later. She received 2000 rad of whole-abdomen radiation and 4000 rad to the supraclavicular, mediastinal, abdominal, para-aortic, and pelvic regions. She is without evidence of disease 72 months since initial diagnosis. Two other patients with Stage II and III dysgerminoma were treated with external radiation therapy and were without evidence of disease at 12 and 64 months of follow-up. The remaining three patients with dysgerminoma presented with Stage IA disease and were treated with operation alone. They remain without evidence of disease at 66, 67, and 141 months.

Toxicity. The toxicity seen in the 27 patients who received chemotherapy was acceptable. Transient alopecia was the most common adverse effect noted with the VAC regimen. Four of the 23 patients treated with VAC experienced transient peripheral neuropathies. The vincristine dose was decreased thereafter in these patients. Severe leukopenia occurred in three patients who received VAC. One of these patients developed a fever of indeterminate etiology and received 6 days of gentamicin and clindamycin therapy without sequelae. A second patient with severe leukopenia developed a staphylococcal folliculitis which was successfully treated. The third patient with severe leukopenia had no clinical manifestations or complications. Significant transient stomatitis was noted in one patient who received VAC. Severe nausea and vomiting were noted in several patients treated with VAC; however, therapy was not interrupted by this. We have used methylprednisolone (Solu-Medrol) as an antiemetic agent for the past several years.

One of the two patients treated with a Cytoxan-5fluorouracil-actinomycin D regimen had an episode of sepsis that was managed without problem. The two patients treated with the VBP regimen tolerated therapy without complications.

The 36 patients have been followed up for 21 to 141 months from the time of diagnosis, with a median follow-up of 68 months. The median follow-up times are 71 months for patients with immature teratoma, 87 months for patients with endodermal sinus tumor, 91 months for patients with dysgerminoma, and 43 months for patients with mixed germ cell tumor. The two deaths were at 19 and 21 months from the time of initial diagnosis.

Comment

The prognosis for patients with ovarian germ cell malignancies has improved dramatically since the introduction of multiple-agent chemotherapy. The effectiveness of the VAC and VBP regimens has been demonstrated in this and other studies. 9-13 The duration of chemotherapy, role of second-look laparotomy, and relative effectiveness and toxicity of the VAC and VBP. regimens are current areas of discussion.

The University of California (Irvine) Department of Obstetrics and Gynecology, Division of Gynecologic Oncology, presently recommends nine courses of VAC chemotherapy for all patients with Stage IA, grade 2 and grade 3 immature teratoma and for all patients with immature teratoma of more advanced stages (Table III). We are currently investigating shortening the duration of therapy to six courses. Stage IA, grade 1 tumors have a favorable prognosis without chemotherapy, but treatment may be reasonable in patients with large tumors. Second-look laparotomies are performed upon the completion of chemotherapy. Patients with immature teratoma and failure of VAC chemotherapy are treated with the VBP regimen.

Patients with endodermal sinus tumor or mixed germ cell tumor are currently treated with VAC for nine courses or VBP for four courses, with a preference for VBP if disease is very advanced. 14-16 Patients with mixed germ cell tumor including dysgerminoma are treated with the VBP regimen in view of the success of cisplatincontaining regimens in the treatment of seminomas.

Patients with failure of VBP or VAC are treated with the other regimen. Patients who have failure of one multiple chemotherapy regimen can be salvaged with a second multiple-agent regimen. Patients with endodermal sinus tumor and mixed germ cell tumor undergo second-look laparotomy at the completion of therapy. Tumor markers may be useful in assessing the response of endodermal sinus tumor to therapy, but the presence of dysgerminoma or immature teratoma can only be assessed histologically. Diagnosing persistent disease early rather than waiting for bulky recurrence may improve the chances of response to second-line agents.

Patients with Stage IA dysgerminomas are carefully observed. Patients with recurrences and more advanced disease are treated with external radiation therapy. Cisplatin-containing regimens are used for patients who have failure of radiation therapy.

All patients with malignant ovarian germ cell tumor should be screened at the time of initial operation for the presence of serum hCG and α-fetoprotein. If these tumor markers are present they should be evaluated serially. Careful histologic examination of the primary tumor and all implants is essential. The presence of sarcoma or other malignant elements is likely to deastically worsen the prognosis.

Conservative surgical management with preservation of fertility is reasonable in these young patients. The majority of these tumors are Stage IA at diagnosis. Unilateral salpingo-oophorectomy, careful inspection of the contralateral ovary, pelvic sidewalls and paraaortic regions, pelvic and abdominal washings, and partial omentectomy are our recommended primary surgical procedures in the young patient with Stage IA disease. Patients with apparent Stage IA dysgerminomas also undergo selective unilateral pelvic/para-aortic lymphadenectomy or postoperative lymphangiography. Patients with Stage III nondysgerminomatous mulignant ovarian germ cell tumors and a normal uterus and contralateral ovary can also be managed with umlateral adnexectomy with removal or biopsy of all meastatic nodules. These tumors are large, and vertical abdominal surgical incisions are essential for accurate staging and atraumatic removal of these tumors.

Multiple-agent chemotherapy has dramatically improved the prognosis of patients with nondysgemninomatous ovarian germ cell malignancies such that in

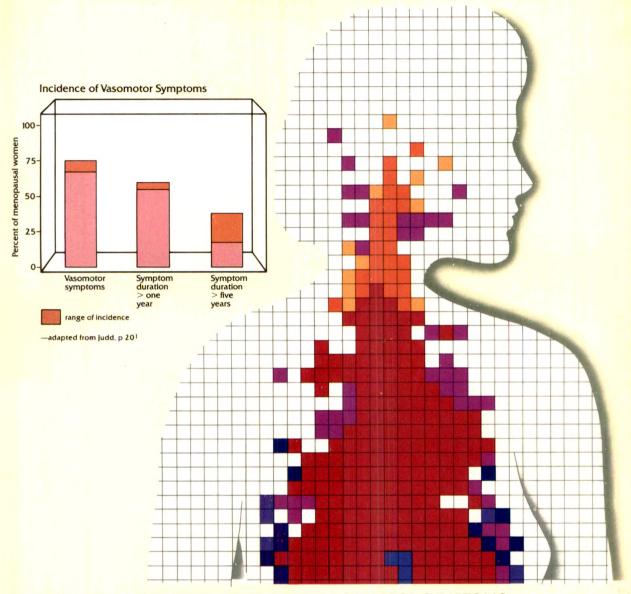
our series survival was independent of prognostic factors including grade and stage of disease. The optimal duration of chemotherapy and relative effectiveness and toxicities of the VAC and VBP regimens are current areas of research.

We wish to thank Judy Sacco, Hazel Hernandez, and Maureen Leyva, R.N., for their invaluable assistance.

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VASOMOTOR SYMPTOMS THAT DEMAND INTERVENTION



PREMARIN RELIEVES MODERATE TO SEVERE VASOMOTOR SYMPTOMS

Vasomotor symptoms are the most common manifestation of the menopause, affecting up to 75% of menopausal women. Of these, 80% may suffer for more than a year and up to 50% for more than five years. These symptoms can disrupt a woman's life by chronically interrupting sleep, resulting in anxiety and irritability.

In a study of postmenopausal women suffering severe episodes of cutaneous flushing, symptoms improved markedly after administration of estrogen²—the treatment of choice for moderate to severe vasomotor symptoms. The estrogen of choice is PREMARIN, the most widely prescribed estrogen for over 40 years. PŘEMARIN (Conjugated Estrogens Tablets, U.S.P.) relieves moderate to severe vasomotor symptoms of the natural menopause, as well as the acute and often severe symptoms of surgical menopause.

(CONJUGATED ESTROGENS TABLETS, U.S.P.)







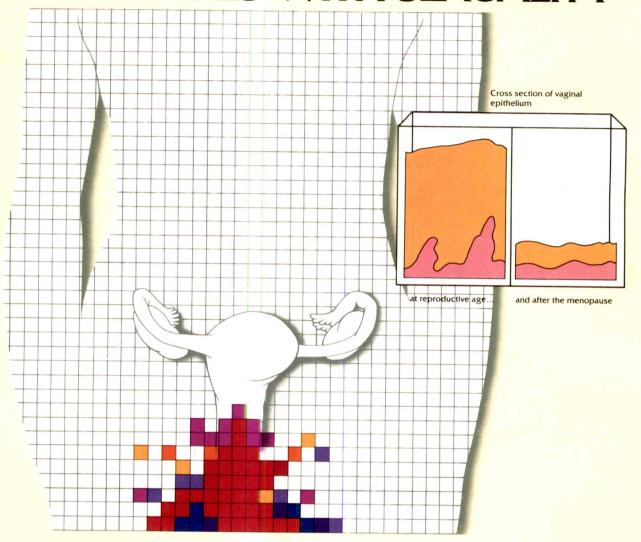




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VAGINAL ATROPHY THAT INTERFERES WITH SEXUALITY



PREMARIN RESTORES THE VAGINAL ENVIRONMENT

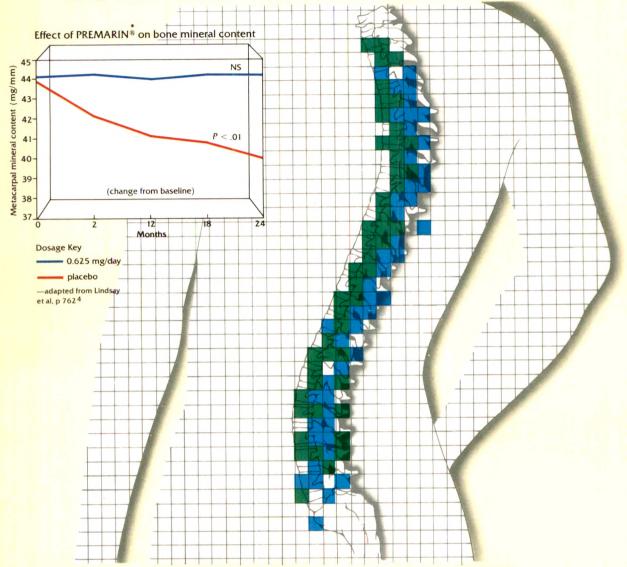
In the postmenopausal woman, decreasing levels of estrogen can have devastating effects on a woman's sexual functioning. The pH of vaginal secretions rises, promoting the growth of contaminating organisms. The vaginal epithelium dries and thins, becoming susceptible to irritation, injury, and infection. Sexual relations may be difficult or impossible.

PREMARIN (Conjugated Estrogens, U.S.P.) Vaginal Cream focuses therapy at the site of the problem. Vaginal dryness is relieved, pH reverts to its normal acidity, and the epithelium thickens and becomes more resistant to injury and infection. With the vaginal environment returned to its premenopausal state, sexual function may improve.

PREMARIN® (CONJUGATED ESTROGENS, U.S.P.) Vaginal Cream



POSTMENOPAUSAL BONE LOSS THAT INCAPACITATES



PREMARIN MAY HALT THE DISABLING COURSE OF OSTEOPOROSIS*

Osteoporosis has an enormous epidemiological impact: it affects 10 million American women, and 26% of all women over age 60.5 The disease begins silently and progresses inexorably for 15 to 20 years, until disabling complications occur.6

To minimize osteoporotic damage, the condition must be detected early and treated promptly. For many patients, PREMARIN is optimal therapy for osteoporosis, as part of a comprehensive program that includes exercise, good nutrition, and calcium supplements. In a controlled study of postmenopausal and oophorectomized women, PREMARIN (Conjugated Estrogens Tablets, U.S.P.) doses of 0.625 mg/day prevented loss of metacarpal mineral content (see graph above).4

PREMARIN® (CONJUGATED ESTROGENS TABLETS, U.S.P.)











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*Conjugated Estrogens Tablets have been evaluated as probably effective for estrogen-deficiency-induced osteoporosis.

Please see last page for brief summary of full prescribing information.

PACKAGE CIRCULARY
PREMARIN® Brand of Conjugated Estrogens Tablets, U.S.P.
PREMARIN® Brand of Conjugated Estrogens, U.S.P. Vaginal Cream in a nonliquefying base

ESTROGENS HAVE BEEN REPORTED TO INCREASE THE RISK OF ENMODMETRIAL ARCINOMA.

1. ESTROGENS HAVE BEEN REPORTED TO INCREASE THE RISK OF ENPOWEETRIAL CARCINOMA.

Three independent case control studies have reported an increased risk of endometimal cancer in postmenopausal women exposed to exogenous estrogens for more than one year. This risk was independent of the other known risk factors for endometrial cancer. These studies are further supported by the finding that incidence rates of endometrial cancer have increased sharply since 1969 in eight different areas of the United States with population-based cancer reporting systems, an increase which may be related to the rapidly expanding used --stogens during the last decade. The three case control studies reported that the risk of endometrial cancer in estrogen users was about 4.5 to 13.9 times greater than in nonusers. There's appears to depend on both duration of treatment and on estrogen dose. In view of these findings, when estrogens are used for the treatment of menopausal symptoms, the lowest dose that writ control symptoms should be utilized and medication should be discontinued as soon as possible. When prolonged treatment is medically indicated, the patient should be reassesseen at least a semiannual basis to determine the need for continued therapy. Although the evidence insust be considered preliminary, one study suggests that cyclic administration of low dose: aftestrogen may carry less risk than continuous administration; it therefore appears prudent to affilize such a regimen. Close clinical surveillance of all women taking estrogens is important. It as access of undagnosed persistent or recurring abnormal vaginal bleeding, adequate diagnostic measures should be undertaken to rule out malignancy. There is no evidence at present that "natural" estrogens are more or less hazardous than "synthetic" estrogens at equiestrogenic doses.

2. ESTROGENS SHOULD NOT RE USED DURING PREGNANCY.

sures should be undertaken to rule out malignancy, there is no evidence at pleasant trial matural estrogens are more or less hazardous than "synthetic" estrogens at equiestrogenic doses.

2. ESTROGENS SHOULD NOT BE USED DURING PREGNANCY.

The use of female sex hormones, both estrogens and progestogens, during earlinges of the strogens are progestogens, during earlinges of the strogen and service of the strogens are increased risk of developing in later to to diethylstilbestrol, a non-steroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. This risk has been estimated as not greater than 4 per 1000 exposures. Furthermore, a high percentage of such exposed women (from 30 to 90 percent) have been found to have vaginal adenosis, epithelial changes of the vaginal and cervix. Although these changes are histologically benign, it is not known whether they are precursors of malignancy. Although similar data are not available with the use of other estrogens, it cannot be presumed they would not induce similar changes. Several reports suggest an association between intrauterine exposure to female sex hormones and bargenital anomalies, including congenital heart defects and limb reduction defects. One mase control study estimated a 4.7-fold increased risk of limb reduction defects in infants exposed in alteroto sex hormones (oral confraceptives, hormone withdrawal tests for pregnancy, or attempted treatment for threatened abortion). Some of these exposures were very short and insorted the propestional pregnancy or attempted treatment for threatened abortion). Some of these exposures were very short and insorted and a few days of treatment. The data suggest that the risk of limb reduction defects in exposed fetuses is somewhat less than 1 per 1000. In the past, female sex hormones have been used during pregnancy or in an attempt to treat threatened or habitual abortion. There is coministerable evidence that estrogens are ineffective for t

DESCRIPTION: PREMARIN (Conjugated Estrogens, U.S.P) contains a mixture of estrogens obtained exclusively from natural sources, blended to represent the average composition of material derived from pregnant mares' urine. It contains estrone, equilin, and 17α -dihydroecus model the with smaller amounts of 17α -estradiol, equilenin, and 17α -dihydroequilenin as salts of their sulfate esters

INDICATIONS: Based on a review of PREMARIN Tablets by the National Attachmy of Sciences—National Research Council and/or other information, FDA has classified the indications for use as follows:

Effective: I. Moderate to severe vasomotor symptoms associated with the menopals set. [There is no evidence that estrogens are effective for nervous symptoms or depression, without associated vasomotor symptoms, and they should not be used to treat such conditions.]

Related vasomotor symptoms, and they should not be used to treat such conderens.)

Atrophic vaginitis

Kraurosis vulvae

Female hypogonadism

Female castration

Primary ovarian failure

Breast cancer (for palliation only) in appropriately selected women and men with metastatic disease.

metastatic disease.

8. Prostatic carcinoma – palliative therapy of advanced disease.

9. Postpartum breast engorgement – Although estrogens have been widely used for the prevention of postpartum breast engorgement, controlled studies have demonstrated hat the incidence of significant painful engorgement in patients not receiving such hormone; therapy is low and usually responsive to appropriate analgesic or other supportive therapy. Considerapy the benefit to be derived from estrogen therapy for this indication must be carefully weighed against the potential increased risk of puerperal thromboembolism associated with Feruse of large doses of estrogens.

PREMARIN HAS NOT BEEN SHOWN TO BE EFFECTIVE FOR ANY PUR POSE DURING PREGNANCY AND ITS USE MAY CAUSE SEVERE HARM TO THE FETUS (SEE GOXED WARNING).

**Probably* effective: For estrogen deficiency-induced osteoporosis, and only when used in conjunction with other important therapeutic measures such as ciet, calcium, physiciherapy, and good general health-promoting measures. Final classification of this indicatien requires further investigation.

and good general health-promoting measures. Final classification of this indication requires further investigation.

INDICATIONS: PREMARIN (Conjugated Estrogens, U.S.P.) Vaginal Cream is indicated in the treatment of atrophic vaginitis and kraurosis vulvae. PREMARIN Vaginal Cream IHES 80° BEEN SHOWN TO BE EFFECTIVE FOR ANY PURPOSE DURING PREGNANCY AND ITS US: MAY CAUSE SEVERE HARM TO THE FETTUS (SEE BOXED WARNING).

CONTRAINDICATIONS: Estrogens should not be used in women (or men) with any olether following conditions: 1. Known or suspected cancer of the breast except in appropriately selected patients being treated for metastatic disease. 2. Known or suspected estrogen-dependeat neoplasia. 3. Known or suspected pregnancy (See Boxed Warning). 4. Undiagnosed abnormal genetal electing. 5. Active thrombophibbits or thromboembolic disorders associated with previous estrogen use (except when used in treatment of breast or prostatic malignancy).

WARNINGS: Long term continuous administration of natural and synthetic estrogensials certain animal species increases the frequency of carcinomas of the breast, cervix, vagina, and liver. There are now reports that estrogens increase the risk of carcinoma of the endometrium in flumens. (See Boxed Warning). At the present time there is no satisfactory evidence that estrogens given to postmenopausal women increase the risk of carcinoma of the endometrium in flumens. (See Boxed Warning). At the present time there is no satisfactory evidence that estrogers given to postmenopausal women increase the risk of carcinoma of the endometrium in flumens. (See Boxed Warning). At the present time there is no satisfactory evidence that estrogers given to postmenopausal women by the press of the properties and properties of the properties of t

pulmonary embolism and thrombophlebilis. When doses of this size are used, any of the thromboembolic and thrombophlebilis. When doses of this size are used, any of the thromboembolic and thromboth adverse effects should be considered a clear risk.

Benigh hepatic adenomas should be contisted in estrogen users having abdominal pain and tenderness, abdominal mass, or hypowork. Hepatocellular carcinoma has been reported in women taking estrogen-containing oral contrates. Increased blood pressure may occur with use of estrogens in the menopause and blood pressure monitored with estrogen use A worsening of glucose tolerance has been observed in pulled some monitored with estrogen use A worsening of glucose tolerance has been observed in pulled by the contracting or the contracting of the pulled by the contracting of the con

2. Given cyclically: Female hypogonausin. Female coadcolors.

Female hypogonadism — 2.5 to 7.5 mg daily, in divided doses for 20 days, followed by a rest period of 10 days: duration. If bleeding does not occur by the end of this period, the same dosage schedule is repeated. The number of courses of estrogen therapy necessary to produce bleeding may vary depending on the responsiveness of the endometrium.

If bleeding occurs before the end of the 10 day period, begin a 20 day estrogen-progestin cyclic regimen with PREMARIN (Conjugated Estrogens Tablets, U.S.P.), 2.5 to 7.5 mg daily in divided doses, for 20 days. During the last five days of estrogen therapy, give an oral progestin. If bleeding occurs before this regimen is concluded, therapy is discontinued and may be resumed on the lifth day of bleeding.

before this regarder as control belief in the primary ovarian failure —1.25 mg daily, cyclically. Adjust upward or downward according to response of the patient. For maintenance, adjust dosage to lowest level that will provide effective control.

Otherwise the retard propression)—1.25 mg daily, cyclically.

will provide effective control.

Osteoporosis (to retard progression) = 1.25 mg daily, cycl-cally.

3. Given for a few days: Prevention of postpartum breast engorgement = 3.75 mg every four hours for five doses, or 1.25 mg every four hours for five days.

4. Given chronically. Inoperable progressing prostatic cancer = 1.25 to 2.5 mg three times daily, Inoperable progressing breast cancer in appropriately selected men and postmenopausal women = 10 mg three times daily for a period of at least three months.

Patients with an intact uterus should be monitored for signs of endometrial cancer and appropriate measures taken to rule out malignancy in the event of persistent or recurring abnormal vaginal bleeding.

bleeding PREMARIN* Brand of Conjugated Estrogens, U.S.P. Vaginal Cream Given cyclically for short-term use only. For treatment of atrophic vaginitis or kraurosis vulvae. The lowest dose that will control symptoms should be chosen and medication should be discontinued as promptly as possible. Administration should be cyclic (e.g., three weeks on and one week off). Attempts to discontinue or taper medication should be made at three to six month intervals. Usual dosage range: 2 to 4 g daily, intravaginally or topically, depending on the severity of the condition.

condition. Treated patients with an intact uterus should be monitored closely for signs of endometrial cancer and appropriate diagnostic measures should be taken to rule out malignancy in the event of persistent or recurring abnormal vaginal bleeding. HOW SUPPLIED: PREMARIN (Conjugated Estrogens Tablets, U.S.P.). No. 865 — Each purple tablet contains 2.5 mg in bottles of 100 and 1,000. No. 866 — Each yellow tablet contains 1.25 mg in bottles of 100 and 1,000. Also in Cycle Pack of 21 and in unit dose package of 100. No. 864 — Each white tablet contains 0.9 mg in bottles of 100. Also in Cycle Pack of 21. No. 867 — Each maroon tablet contains 0.625 mg in bottles of 100. Also in Cycle Pack of 21. No. 867 — Each maroon tablet contains 0.625 mg in bottles of 100 and 1,000. Also in Cycle Pack of 21 and unit dose package of 100. No. 868 — Each green tablet contains 0.3 mg in bottles of 100 and 1,000. The appearance of these tablets is a trademark of Ayerst Laboratories.

Each green labelet contains u.s. mg in bottes of IOO and 1,000. The appearance of these tablets is a trademark of Ayerst Laboratories.

PREMARIN (Conjugated Estrogens, U.S.P.) Vaginal Cream – No. 872 – Each gram contains 0.625 mg Conjugated Estrogens, U.S.P. (Also contains cetyl esters wax, cetyl alcohol, white wax, glyceryl monostearate, propylene glycoryl monostearate, methyl stearate, phenylethyl alcohol, sodium lauryl sulfate, glycerin, and mineral oil.)

Combination package: Each contains Net Wt. 1½ oz. (42.5 g) tube with one calibrated plastic applicator.

applicator. Also Available - Refill package: Each contains Net Wt. $1V_2$ oz. (42.5 g) tube.

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Use of femur length in estimation of fetal weight

Lyndon M. Hill, M.D., Robert Breckle, R.T., R.D.M.S., William C. Gehrking, R.T., and Peter C. O'Brien, Ph.D.

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Fetal weight was estimated sonographically within 3 days of delivery in 103 cases. A stepwise regression analysis was performed to evaluate fetal sex, biparietal diameter, head circumference, abdominal circumference, and femur length as factors in estimation of fetal weight. A new formula for calculating fetal weight was derived. Fetal sex did not affect the results obtained. Incorporation of femur length improved the reliability of the weight estimate. Because of intrapopulation and interpopulation differences, institutions using obstetric ultrasound examination techniques should establish their own formulas for estimating fetal weight. (AM J OBSTET GYNECOL 1985;152:847-52.)

Key words: Ultrasound, femur length, estimated fetal weight

Numerous fetal body dimensions that can be measured by ultrasonography have been evaluated in an attempt to estimate fetal weight more reliably. For example, Suzuki et al.1 used fetal heart volume and Thompson et al.2 used the diameter of the thorax to estimate fetal weight. Campbell and Wilkin³ estimated fetal weight from the circumference of the fetal abdomen and claimed that 95% of birth weights fell within 106 gm of the estimate. Use of various body diameters in conjunction with the biparietal diameter has been found to increase the accuracy of the ultrasonographic prediction of fetal weight. One of the more commonly used formulas incorporates ultrasonographic measurements of abdominal circumference and biparietal diameter.1 Some methods that have been developed require complex mathematical equations and computer analysis.5

Since femur length closely correlates with crown-heel length,⁶ it has recently been proposed⁷ as an adjunctive measurement in the estimation of fetal weight. The purpose of this investigation was to determine whether the incorporation of femur length could improve the accuracy of fetal weight estimation based upon either abdominal circumference³ or abdominal circumference and biparietal diameter.⁴

Methods

A thorough obstetric ultrasonographic examination was performed on 103 patients within 72 hours before delivery; a linear-array real-time system (Picker, model

LS-2000 linear scanner) with a 3.5 MHz transducer was

In these 103 patients the duration of gestation was as follows: 50 patients, between 38 and 40 weeks; 26, between 35 and 37 weeks; 20, between 32 and 34 weeks; 3, between 29 and 31 weeks; 3, between 26 and 28 weeks; and 1, at 25 weeks' gestation.

The biparietal diameter was obtained at the level of the thalami and septum pellucidum. Measurements were made from the outer edge of the anterior skull table to the inner margin of the posterior skull table. Head circumference was calculated from the same axial image used to measure biparietal diameter. The abdominal circumference was measured at the level of the portal sinus. The femur length was measured from the greater trochanter to the distal metaphysis. Biparietal diameter, femur length, and abdominal and head diameters were initially measured with electronic calipers calibrated to the velocity of sound in tissue, 1540 m/sec. Each measurement was then checked manually with calipers before the data were entered for analysis.

Circumferences were obtained from the formula for the circumference of an ellipse:

$$4.443 \left(\frac{D_1^2 + D_2^2}{2} \right)^{1/2}$$

in which D₁ and D₂ are the semi-axes.

Weight was calculated by using abdominal circumference as described by Campbell and Wilkin,³ abdominal circumference and biparietal diameter as described by Shepard et al.,⁴ and biparietal diameter, abdominal circumference, and femur length as described by Hadlock et al.⁷ Also, a new formula for the estimation of fetal weight was derived from the data at hand.

Standards for birth weight have taken into consideration the approximately 150 gm difference in weight

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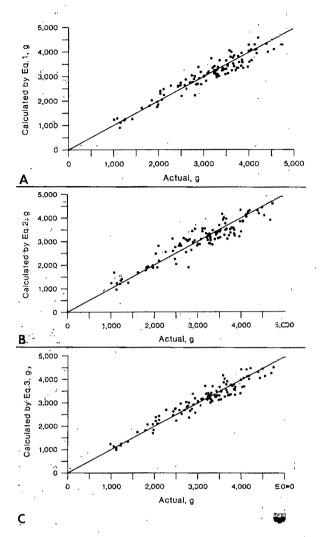


Fig. 1. Calculated fetal weight plotted against actual birth weight. Calculation was by three different equations: A, pased on biparietal diameter, abdominal circumference, and femur length (equation 1); B, based on abdominal circumference (equation 2); C, based on biparietal diameter, femur length, and abdominal circumference with In birth weight as dependent variable.

between males and females near term. Because fetal sex now can be determined with a high degree of accuracy, we sought to include this information in our assessment of fetal weight.

Within 15 minutes after delivery, the neonates were weighed on a metric scale to provide an actual weight for comparison with the calculated weight. The difference between the two was recorded as error in grarts. The percentage error was calculated as follows: % error = $100 \times$ (actual weight – calculated weight). (actual weight).

Results

A stepwise regression analysis, performed after preliminary inspection of the data, indicated that accurate prediction could be accomplished by use of no more

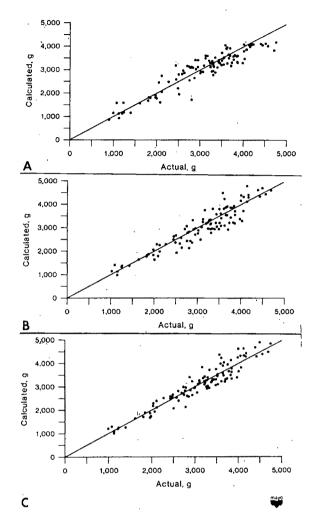


Fig. 2. Calculated fetal weight plotted against actual birth weight. Calculation was by three different methods: *A*, the method of Campbell and Wilkin³; *B*, the method of Shepard et al.⁴; *C*, the method of Hadlock et al.⁷

than three coefficients in the prediction equation. An R² analysis was performed to select the best subset of variables. Head circumference did not improve the prediction of fetal weight. Hence it was not considered further.

The variables that gave the best equations with one, two, and three coefficients were as follows:

FL · AC;
$$R^2 = 0.898$$

AC · BPD, FL/BPD; $R^2 = 0.915$
Sex, AC · BPD, FL/BPD; $R^2 = 0.919$

in which FL = femur length, AC = abdominal circumference, and BPD = biparietal diameter. Knowing the fetal sex did not significantly improve the estimation of fetal weight; three equations were developed (Fig. 1). The two-coefficient equation was selected for further study, and linear regression analysis produced the following formula for birth weight (BW):

Present study Campbell and Shepard Hadlock Wilkin³ et al. Equation 3 Equation 1 Equation 2 Percentile 27.0 34.2 50.0 99.9 10 23.6 43.6 88.2 55.8 62.1 85.3 49.5 20 65.5 109.6 131.0 30 108.0 118.5 70.0 114.7 148.4 124.9 160.9 170.8148.6 145.0 40 206.9 220.0 224.0 167.6 184.1 193.1 50 252.4 278.0 258.0 216.1 60 215.1277.3342.6 329.6 296.4 273.6 70 283.6 329.1 374.8 441.6 446.6 413.6 359.3 80 357.8 605.2 400.2 485.5 529.8 90 411.7

Table I. Percentiles for absolute errors in estimating fetal weight by six different equations

$$BW = -3153.1 + 13.645 (AC \cdot BPD) + (1)$$

2753.97 (FL/BPD)

A second equation was obtained from a linear regression on abdominal circumference:

$$BW = -2883.6 + 181.39 \text{ (AC)} \tag{2}$$

The analysis was then rerun with 1n birth weight as a dependent variable rather than birth weight, and this stepwise regression provided the following equation:

$$BW = \exp \left[-4.7208 + 1.1933 \text{ (BPD)} - (3) \right.$$

$$0.0613 \text{ (FL} \cdot \text{BPD)} + 5.9509 \text{ (FL/BPD)} +$$

$$0.3339 \text{ (AC/BPD)}$$

for which $R^2 = 0.946$.

In Fig. 2, actual birth weights are plotted against fetal weights calculated from the equations of Campbell and Wilkin, Shepard et al., and Hadlock et al. The relative ranking of the six equations, from those least able to predict birth weight to those most able, was as follows: (1) Campbell and Wilkin; (2) Shepard et al.; (3) our equation 2; (4) Hadlock et al.; (5) our equation 1; (6) our equation 3. The statistically significant ($p \le 0.05$) differences were our equation 1 versus all other methods except the method of Hadlock et al. and our equation 3 versus all other methods. Thus our equation 3 was significantly superior to all of the five other equations.

Table I gives the percentiles for the absolute errors. With use of equation 3, 50% of the estimated fetal weights were within 168 gm of actual birth weight, and with the method of Shepard et al., 50% were within 224 gm. Overall, we were within 605.2 gm 90% of the time with Shepard's formula, compared with 400.2 gm with equation 3.

Comment

The primary purpose of our study was to evaluate the effect of a number of different fetal variables on estimations of weight in utero.

Fetal sex. The accuracy of identification of fetal sex

has been reported to be as high as 98.4% after 24 weeks of gestation. However, this factor did not significantly affect fetal weight estimation in the present study. In an analysis of 52,004 single births in the city of Aberdeen, Thomson et al. found no consistent sex difference in weight until 34 to 35 weeks of gestation; after 38 weeks, males were 150 gm heavier than females. The available data to 35 meavier than females, the ultrasonographic features used to estimate fetal weight reflect these differences.

Biparietal diameter. Biparietal diameter was one of the first fetal measurements to be used in weight estimation. Most published formulas, however, were linear functions and had standard deviations of approximately 500 gm.^{2, 13, 14} With use of nonlinear functions, a slight improvement may be obtained.¹⁵ Use of the abdominal circumference with biparietal diameter has been found to improve the accuracy of fetal weight estimation.^{4, 16, 17} Eik-Nes et al.¹⁸ noted that as the biparietal diameter increases in size relative to the abdomen, so does its effect upon fetal weight estimation. Thus the fetal weight of a heavy fetus with a relatively small biparietal diameter would be underestimated.

Abdominal circumference. Campbell and Wilkin³ and Campogrande et al.¹⁹ have shown that abdominal circumference alone is a good predictor of fetal weight. Higginbottom et al.²⁰ reported the mean error in predicting fetal weight from abdominal circumference to be 75 gm; in 94% (47 of 50 cases), the error was less than 145 gm. Our results confirm the close correlation between abdominal circumference and fetal weight. The additional use of other fetal measurements (biparietal diameter and femur length) significantly improves the estimation of birth weight.

Head circumference. Head circumference is considered by some²¹ to be a more reliable indicator of gestational age, particularly in the last 6 weeks of pregnancy when molding is more frequent. Because the shape of the head will affect the biparietal diameter,²² head circumference might also be a more accurate mea-

Table II. Actual and estimated fetal weights from standardized fetal measurements

				•	Fetc	ıl weight (gm)		,
						Estin	ated	
Gestational age (wk)	Biparietal diameter (cm)	Abdominal circum- ference ²⁷ (cm)	Femur length ²⁸ (cm)	$Actual^{29}$ $(n = 40,000)$	Campbell and Wilkin³ (n = 140)	Higgin- bottom et al. 20 $(n = 50)$	Shepard et al. ¹ (n = 73)	Hadlock et al. ⁷ (n = 167)
28	7.0	25.3	5.3	1118	1571	1321	1282	1373
29	7.3	26.9	5.6	1275	1871	1588	1348	1622
30	7.5	22.4	5.8	1458	1970	1679	1429	1744
31	7.7	28.0	6.0	1648	2090	1791	1623	1873
32	7.8	28.7	6.2	1861	2232	1929	1715	2008
33	8.1	29.0	6.4	2095	2294	1990	2020	2149
34	8.4	30.1	6.6	2298	2523	2225	2255	2369
35	8.5	32.2	6.8	2489	2958	2724	2387	2758
36	8.8	33.3	7.0	2697	3178	3013	2635	3101
37	8.9	34.4	7.2	2960	3387	3321	2937	3372
38	9.0	35.7	7.4	3171	3614	3712	3145	3655
39	9.2	35.9	7.6	3325	3647	3776	3353	3845
40	9.4	36.1	7.8	3448	3679	3839	3538	3937
41	9.6	37.1	8.0	3547	3828	4167	3751	4234

surement for estimating fetal weight. Hadlock et al. 22 evaluated head circumference as a predictor of menstrual age and, in general, found it to have wider fluctuations than biparietal diameter. When the fetal skull is dolichocephalic or brachycephalic, however, biparietal diameter is no longer an accurate assessor of gestational age. Breech presentation, severe oligohydramnios, and descent of the fetal vertex into the pelvis are situations in which the reliability of the biparietal diameter is impaired. Also, errors in fetal weight estimation might occur if the biparietal diameter is used without regard to head shape. Although the cephalic index22 may be used in most instances to assess the shape of the calvaria, careful scanning of the fetal head should be performed to exclude abnormalities in shape not detected in the plane of the biparietal diameter.23 Preliminary inspection of our data indicated that use of head circumference did not improve fetal weight prediction beyond what was available from other measurements. This agrees with the data of Hadlock et al.7 However, Jordaan24 found that abdominal circumference and head circumference not only estimated fetal weight (mean deviation, -1.07%) more accurately but also prevented gross errors when the head shape was unusual.

Two important points should be considered. First, head circumference was not available for every patient in our study, since at later gestational ages the fetal head was too large to be totally included on a real-time image. Second, for patients with a head circumference measurement, the cephalic index was within the normal range (74% to 83%). Consequently, head circumference may offer an advantage to fetal weight estimation in specific cases, but its incorporation into the formula is

not essential. In instances of dolichocephaly or brachycephaly the biparietal diameter for the head circumference assessment of gestational age can be used in an attempt to reduce the error in fetal weight assessment.

Femur length. Ylöstalo and Järvinen²⁵ incorporated the distance from the base of the skull to the end of the rump (shoulder-to-rump distance) as well as the biparietal diameter and transthoracic diameter in their equation for estimating fetal weight. However, they noted that the determination of fetal body dimensions may be quite difficult. In addition, determination of the shoulder-to-rump length required the use of static rather than real-time scanning-a definite drawback in 1984 when the majority of obstetric sonographic examinations are performed with real-time. Picker and Saunders²⁶ also used the compound gray-scale scanner to obtain the distance from the tip of the fetal head to the inferior extent of the fetal bladder. A fetal body volume could then be obtained. These authors also included femur length and limb width in their equations in order to obtain an estimation of limb volume. Jordaan²⁴ found that birth weight increased with crown-heel length when abdominal circumference was held constant. Hence the weight of very short fetuses was overestimated, and the weight was underestimated in those who were very long. Hadlock et al.7 found a strong linear relationship between femur length and crown-heel length and hence incorporated the former measurement into their estimation of fetal weight. Our results also demonstrate that weight estimates incorporating femur length are the most accurate. Although the observed differences in accuracy between equation 3 and equation 1 are statistically significant and therefore not attributable to chance, the magnitude of the improvement obtained with equation 3 is not large (relative to equation 1). Both equations are easily computed with a hand-held calculator.

The relationship between weight and the linear fetal measurements obtained ultrasonographically provides significant improvement in the estimates of weight, but there is an irreducible inherent error. The mere act of measuring introduces error that is compounded as the number of fetal variables assessed increases. Individual variations both within and between populations must also be considered. In studies based on relatively few patients, sampling errors may arise if the few patients studied are not truly representative of the general population.

Table II compares the fetal weight estimates from four separate studies with the results of Babson et al., 29 who tabulated neonatal weight at specified gestational ages in more than 40,000 deliveries. There is no apparent trend or association between the weights obtained from a specific biparietal diameter, abdominal circumference, and femur length. However, the investigators used different populations to obtain their data. Since each paper reported a reasonably close correlation between the estimated weight and birth weight, fetal anthropomorphic characteristics must be considered as giving rise to some of the differences obtained. Depending on the population studied, the ratio of limb to trunk and the distributions of fat, muscle, and bone in the neonatal body differ. As noted by Jordaan²⁴ and reported by Falkner,30 these differences are already present in the fetus. Blacks, for example, tend to have a shorter trunk and longer forearms.31 Hence, although a formula may be accurate for the population from which it was derived, it may not be generally applicable because of interpopulation differences. Therefore, each obstetrics department should evaluate its own population and obtain appropriate data. Previously published equations for estimating fetal weight may then be assessed. If the data obtained do not closely fit any of the published equations, a new equation must be derived for that institution. For our population, the incorporation of biparietal diameter, abdominal circumference, and femur length gives a statistically more reliable estimate of fetal weight.

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The effects of α - and β -adrenergic stimulation on contractility and prostaglandin (prostaglandins E_2 and $F_{2\alpha}$ and 6-keto-prostaglandin $F_{1\alpha}$) production of pregnant human myometrial strips

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The effects of catecholamines and α - and β -adrenergic agonists and antagonists on the spontaneous contractility of superfused pregnant human myometrial strips are reported. Prostaglandins (prostaglandins E2 and F2a and 6-keto-prostaglandin F1a) were analyzed in the effluent of the superfusion medium by specific radioimmunoassays. Both epinephrine and norepinephrine dose-dependently (10 ng/ml to 1 μ g/ml) stimulated the contractility of the myometrial strips and significantly increased the synthesis of all prostaglandins assayed. α -Adrenergic blockers inhibited the catecholamine-induced increase in contractility. This was associated with decreased prostaglandins F_{2a} and E_2 concentrations and a further increase in 6-keto-prostaglandin F_{1a} levels. Exclusive β -adrenergic stimulation with β -mimetic drugs had the same effect. Conversely, epinephrine stimulation together with β -blockers resulted in a further increase in the prostaglandins F_{2a} and E_2 release of the myometrial strips. This effect was even more pronounced with specific α -adrenergic stimulant drugs. Our results demonstrate the interrelationship of α - and β -adrenergic stimulation and the prostaglandin system. α -Adrenergic stimulation increases myometrial contractility and the synthesis of prostaglandins F_{2a} and E_2 , β -Adrenergic stimulation recuces contractility by further enhancing 6-keto-prostaglandin F_{1a} production. (AM J OBSTET GYNECOL 1985;152:852-6.)

Key words: Human myometrium, prostaglandins, catecholamines, pregnancy

Various effects of catecholamines on the human myometrium have been described. Epinephrine has been reported to inhibit uterine contractions, to increase them, and to diminish or increase them depending on the dose. It has been shown that the myometrium has two adrenoreceptors, the excitatory α -adrenoreceptor and the inhibitory β -adrenoreceptor and that the response to catecholamines is determined according to which receptor is predominant. In the uteri of various

species, significant quantities of catecholamines have been reported and norepinephrine has been found to be the dominant uterine catecholamine.3.4 The interactions between catecholamines and prostaglandins have been established in several organs, and prostaglandins have been proved to be modulators of the autonomic nervous system. 5 Norepinephrine stimulates prostaglandin (PGE and PGF) synthesis and, conversely, high prostaglandin concentrations inhibit the catecholamine release.5.6 Prostaglandins play a basic role in the regulation of uterine motor activity and their physiologic significance for myometrial contraction and relaxation has been proved.7 The present study investigates the effects of catecholamines and selective αand β-adrenergic agonists and antagonists on the contractility and prostaglandin production of human pregnant myometrium strips.

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Material and methods

Specimens of myometrium obtained during cesarean section deliveries (36 to 42 weeks of pregnancy) from the isthmus uteri were immediately transferred in icecold Tyrode's solution to the laboratory. After preparation the myometrial strips were approximately 20 mm long, 5 mm wide, and 2 mm thick and were fixed in the previously described superfusion chamber. The myometrial strips were continuously superfused with oxygenated Tyrode's solution (pH 7.4, 37° C) at a flow rate of 1 ml/min. After the onset of regular contractions, norepinephrine (Arterenol, Hoechst) and epinephrine (Suprarenin, Hoechst) were added with an infusion pump in rising concentrations (1 ng/ml to 1 $\mu g/ml$).

The other drugs used included the α-blockers phentolamine (Regitine, Ciba-Geigy), prazosin (Minipress, Pfizer), and yohimbine (Yohimbin Spiegel, Kali-Chemie); the \(\beta\)-mimetics or ciprenaline (Alupent, Boehringer Ingelheim) and fenoterol (Partusisten, Boehringer Ingelheim); the β-blocker pindolol (Visken, Sandoz); and the α-stimulator norfenephrine (Novadral, Gödecke).

The effluent of the superfused myometrial strips was collected in 5 ml fractions and immediately stored at -80° C. Without extraction 6-keto-PGF₁₀, PGE₂, and PGF_{2α} were determined by highly specific and sensitive radioimmunoassay systems.7 In preliminary experiments, no differences in the mechanical reactions to adrenergic stimulation were observed in myometrial specimens obtained from week 36 to week 42 of gestation. The results of prostaglandin determination were therefore pooled.

Results

Effects of epinephrine and norepinephrine on the contractility and prostaglandin synthesis of pregnant human myometrial strips. Both epinephrine and norepinephrine stimulated the contractility of myometrial strips (Table I). The frequency of contractions and basal tone increased dose-dependently. Catecholamines in a concentration of 1 ng/ml had no stimulating effect. Beginning at a dose of 10 ng/ml (100 ng/ml) norepinephrine (epinephrine) caused a significant increase in frequency and basal tone with rising concentrations. At high catecholamine concentrations (1 µg/ml) the muscle went into spasm accompanied by highly frequent contractions of low amplitude.

As shown in Tables II to IV, the mean 6-keto-PGF₁₀ concentration in the superfusion medium of the pregnant myometrial strips during spontaneous contráctions was 6.4 times higher (range, 4.8 to 8.2) than the PGF₂₀ level. PGE₂ concentrations were only slightly lower than 6-keto-PGF_{1a} (range, 0.7 to 1.4). Both nor-

Table I. The effect of catecholamines and αor β-adrenergic receptor modulating drugs on myometrial contractions

	Co	ncentrati	on (μg/1	m!)
,	0.001	0.01	0.1	I
Catecholamines	<u> </u>			
Epinephrine	0	0	+	++
Norepinephrine	0	(+)	+	++
α-Stimulator				
Norfenephrine	(+)	+	+	++
α-Blocker				
Phentolamine	0	0	0	(-)
Prazosin	0	0	(-)	-*
Yohimbine	0	0	0	_*
β-Stimulator				
Orciprenaline*	(-)	(-)		_
Fenoterol*	(-)			_
β-Blocker				
Pindolol	0	0	0	(+)
Epinephrine + α-blocker				
Phentolamine†	0	0	(-)	(-)
Prazosir.†	ő	(-)	`_'	`-'
Yohimbine†	ő	`o´	(-)	_
Epinephrine + β-blocker	v	Ü	()	
Pincolol†	.0	(+)	+	+ +

 $^{0 = \}text{No effect}, + = \text{stimulation}, - = \text{inhibition}, () =$ weak reaction.

epinephrine and epinephrine had a significant stimulatory effect on the prostaglandin synthesis. Depending on the dose, PGE2 concentrations increased 3 to 7 times, PGF₂₀ 3 to 11 times, and 6-keto-PGF₁₀ 2 to 4 times (Table II). Accordingly, the PGF_{2α} and 6-keto-PGF_{1α} ratio rose between 0.12 to 0.38 (0.18 to 0.41). The catecholamine-induced increase in PGF_{2n} > PGE₂ > 6keto-PGF₁₀ production corresponded with the contractility-stimulating effect. Significant differences in the stimulatory effect of epinephrine and norepinephrine on contractility and prostaglandin synthesis were not observed.

Effects of α-adrenergic blockade on catecholamineinduced contractility and prostaglandin synthesis and of specific β-adrenergic stimulation. Simultaneous superfusion of epinephrine and the α-adrenergic blocker phentolamine or prazosin and yohimbine significantly inhibited the catecholamine-stimulated increase in contractility (Table I). Preceding superfusion of α-adrenergic blockers in equivalent doses also inhibited the epinephrine effect and significantly reduced the spontaneous activity of the myometrial strips. Exclusive superfusion of β-adrenergic stimulants (orciprenaline, fenoterol) reduced the frequency and amplitude of spontaneous contractions but did not inhibit them completely, even in rising concentrations. During contin-

^{*}No complete inhibition (fading effect).

[†]Effect on epinephrine (1 µg/ml) stimulated contractions.

Table II. Mean PGE₂, PGF_{2a}, and 6-keto-PGF_{1a} ⊃⊐centrations (±SD) in the superfusion medium of gravid human myometrium strips during spontæneous contractions and catecholomine stimulation

	n ·	PGE_2	n	PGF_{2a}	n	6-keto-PGF _{1a}	PGF _{2a} /6-keto-PGF _{1a} ratio
Spontaneous contractions	6	1.46 ± 0.96	6	0.23 ± 0.08	5	1.89 ± 0.31	0.12
Norepinephrine, 100 ng/ml	5	4.32 ± 1.46*	4	$0.80 \pm 0.20*$	· 5	4.13 ± 0.89*	0.19
Norepinephrine, 1 µg/ml	7	9.76 ± 1.32*	8	$2.62 \pm 0.49*$	7	6.93 ± 1.02*	0.38
Spontaneous contractions	7	1.98 ± 0.87	6	0.39 ± 0.17	7	2.19 ± 0.46	0.18
Epinephrine, 100 ng/ml	4	$4.39 \pm 1.24*$	4	$0.97 \pm 0.31*$	4	4.50 ± 0.28*	0.22
Epinephrine, 1 µg/ml	8 ·	$8.13 \pm 2.05*$	9	$2.83 \pm 0.40*$	8	6.77 ± 0.94*	0.41

Concentrations in ng/min/g wet weight.

Table III. Mean PGE_2 , $PGF_{2\alpha}$, and 6-keto- $PGF_{1\alpha}$ \Rightarrow centrations ($\pm SD$) in the superfusion medium of gravid human myometrium strips during sponta eous contractions, epinephrine stimulation, α-adrenergic blockade, and β-adrenergic stimulation

	n	PGE_2	-1	$PGF_{2\alpha}$	n	6-keto-PGF _{1α}	PGF _{2α} /6-keto-PGF _{1α} ratio
Spontaneous contractions Epinephrine, 1 µg/ml Phentolamine, 1 µg/ml + epinephrine, 1 µg/ml	4 4 5	2.03 ± 1.13 4.74 ± 0.91* 1.90 ± 0.54†	5 7	0.27 ± 0.06 $1.32 \pm 0.54*$ $1.13 \pm 0.30†$	4 4 6	1.47 ± 0.15 4.98 ± 0.74* 5.46 ± 0.43*	0.18 0.37 0.21
Spontaneous contractions Epinephrine, 1 µg/ml Prazosin, 1 µg/ml + epinephrine, 1 µg/ml	4 3 4	1.65 ± 0.97 $5.88 \pm 1.08*$ $1.41 \pm 1.21\dagger$	5 5	0.39 ± 0.13 $3.12 \pm 0.54*$ $0.78 \pm 0.23†$	5 5 6	1.87 ± 0.27 $5.10 \pm 0.88*$ $7.81 \pm 1.34*$	0.21 0.61 0.10
Spontaneous contractions Epinephrine, 1 µg/ml Yohimbine, 1 µg/ml + epinephrine, 1 µg/ml	3 4 4	1.49 ± 0.47 $6.58 \pm 0.92*$ $1.07 \pm 0.42†$	1 1 1	0.37 ± 0.15 $2.88 \pm 0.47*$ $0.58 \pm 0.23†$	4 5 4	2.04 ± 0.31 $5.91 \pm 1.21*$ $10.51 \pm 1.81*$	0.18 0.49 0.06
Spontaneous contractions Orciprenaline, 10 ng/ml Fenoterol, 10 ng/ml	5 5	1.63 ± 0.71 2.13 ± 0.60	1 1 3	0.25 ± 0.07 0.31 ± 0.22 0.28 ± 0.10	5 5 4	2.13 ± 0.45 $7.02 \pm 0.81*$ $9.31 \pm 0.74*$	0.12 0.04 0.03

Concentrations in ng/min/g wet weight.

uous superfusion of α - or β -adrenergic agonists, the excitatory or inhibitory effect declined over a p=riod of time. This fading reaction was found in all experiments with adrenergic agonists.

As is shown in Table III, α -adrenergic blockads significantly reduced the epinephrine-stimulated increase in PGE₂ and PGF_{2 α} production, 6-keto-PGF_{1 α} leves remained high and increased even further. As is seen in the ratio between PGF_{2 α} and 6-keto-PGF_{1 α}, prazosic and yohimbine were more effective than phentolatine (0.21 versus 0.10, 0.06). Superfusion of the β -ministics or ciprenaline and fenoterol exclusively stimulated 6-keto-PGF_{1 α} production. The reduction of the PG- $\frac{1}{2}\alpha$ 6-keto-PGF_{1 α} ratio corresponds with the β -ministic in bitory effect and reduction of the spontaneous repometrial contractility.

Effects of β-adrenergic blockade on catecholar-ineinduced contractility and prostaglandin synthesia and of specific α-adrenergic stimulation. Superfusion of the β-adrenergic blocker pindolol had no significant stimulating effect on the frequency and amplitude of the spontaneous contractions. No significant differences in the prostaglandin levels were found (Table IV). Simultaneous superfusion of epinephrine and pindolol further increased PGF_{2 α} and PGE₂ production and resulted in a reversal of the PGF_{2 α}/6-keto-PGF_{1 α} ratio (1.48).

Concentrations of the α -adrenergic agonist norfenephrin as low as 10 ng/ml had a significant stimulatory effect on the spontaneous myometrial activity. Higher concentrations proved to have the same effects as those observed after epinephrine stimulation. This selective α -adrenergic stimulation significantly increased the PGF_{2 α} production rate (PGF_{2 α}/6-keto-PGF_{1 α} ratio of 2.26). PGE₂ and 6-keto-PGF_{1 α} levels remained unchanged (Table IV).

Comment

Opposite effects of epinephrine and norepinephrine on the myometrial activity in situ have been described

^{*}p < 0.05.

^{*}p < 0.05 significant increase.

[†]Significant decrease.

Table IV. Mean PGE₂, PGF₂₀, and 6-keto-PGF₁₀ concentrations (\pm SD) in the superfusion medium of gravid human myometrium strips during spontaneous contractions, epinephrine stimulation, β -adrenergic blockade, and α -adrenergic stimulation

:	n	PGE_2	n	$PGF_{2\alpha}$	n	6-keto-PGF _{1α}	PGF₂α/6-keto-PGF₁α ratio
Spontaneous contractions	. 4	$1.74^{\circ} \pm 0.81$	4	0.23 ± 0.07 .	·5	1.96 ± 0.31	0.14
Pindolole, 1 µg/ml	3	3.01 ± 1.28	4	0.45 ± 0.13	3	2.61 ± 0.47	0.17
Epinephrine, 1 μg/ml	4	$8.99 \pm 2.14*$	3	$2.31 \pm 0.41*$	5	$5.47 \pm 0.91*$	0.42
Epinephrine, 1 μg/ml + pindolole, 1 μg/ml	4	10.28 ± 1.91*	4	$6.84 \pm 1.13*$.4	4.63 ± 1.06	1.48
Spontaneous contractions	4	1.88 ± 0.79	· 4	0.34 ± 0.10	5	2.13 ± 0.40	0.16
Norfenephrine, 1 µg/ml	3	3.14 ± 1.67	-4	$7.83 \pm 1.18*$	5	3.47 ± 0.11	2.26

Concentrations in ng/min/g wet weight.

in the literature. It has been reported that epinephrine inhibits and norepinephrine stimulates contractions of the late pregnant and laboring human uterus. In vitro studies did not show any differences between the catecholamine effects: both epinephrine and norepinephrine stimulated contractions of myometrium strips from the late pregnant human uterus.8 In vivo studies demonstrated that the effect of epinephrine depends on its concentration, that is, in high concentrations it caused uterine activity to diminish. Moreover, it has been shown that epinephrine in low concentrations reduced the effect of intravenously administered oxytocin. A possible explanation for the different in vivo and in vitro results lies in this oxytocin antagonizing effect. In vivo epinephrine diminishes uterine activity by reducing the action of oxytocin. In high concentrations the excitatory effect of epinephrine exceeds the oxytocin antagonism and results in intensifying uterine contractions. A further possible explanation for the inhibitory effect of epinephrine on myometrial activity can be derived from its predominantly β-adrenozeceptor stimulating quality.2 According to this hypothesis epinephrine would also have an inhibitory effect on myometrial contractions in vitro. Moreover, it has been proved that norepinephrine does not affect the uterus as a pure α-adrenergic stimulator but has also strong β-adrenergic stimulating qualities.9 Unlike epinephrine, norepinephrine and dopámine have been found to be the dominant uterine catecholamines,4 and in vivo, both norepinephrine and dopamine increased the contractility of the late pregnant human uterus.10 On the basis of in vitro and in vivo studies, most investigators have attributed the excitatory norepinephrine and epinephrine effect to the stimulation of α-adrenoreceptors, whereas the inhibitory effect has been linked to the stimulation of β-adrenoreceptors.9, 11 According to our results, both catecholamines stimulated contractions of the pregnant myometrial strips. We therefore conclude that in late pregnancy there is a gradual change in the threshold of myometrial adrenergic receptors with consequent predominance of the

 α -adrenergic excitatory effects. The inhibition of the stimulatory effects of both catecholamines by α -adrenergic blockers supports this conclusion.

The dose-dependent stimulatory catecholamine effect on myometrial contractions is correlated with an increased prostaglandin production of the myometrial strips. This increase in prostaglandin biosynthesis by adrenergic stimulation has been found in several organs, and conversely, the regulation of catecholamine release by prostaglandins has also been observed. 5. 12 During catecholamine stimulation, the PGF₂₀ production of the myometrial strips increased and the 5-keto-FGF₁₀/PGF₂₀ ratio significantly decreased. This corresponds with the increase in contractility. Exclusive αadrenergic stimulation had the same effect on myometrial contractility as epinephrine and norepinephrine and significantly increased PGF_{2a} levels, whereas 6 keto-PGF₁₀ and PGE₂ concentrations showed no difference. This indicates that a selective α-agonist is not an adequate stimulus for PGE2 release. Similarly, the selective β-adrer ergic stimulation by orciprenaline and fenoterol did not stimulate PGE2 release. This difference between catecholamines and selective α- or β-agonists has also been found in other organs.12

The simultaneous superfusion of β -adrenergic blocking agents along with catecholamines had an inhibitory effect on contractility and PGF_{2a} and PGE₂ synthesis. However, 6-keto-PGF_{1α} levels remained high and even increased. It has been reported that prostacyclin reduces the spontaneous motility of pregnant human myometrium in vitro and the spasm induced by PGF_{2α}.13 According to our results, selective stimulation of β-adrenoreceptors reduced the contractility and increased 6 keto-PGF_{1α} levels. In a recent study it has been shown that the infusion of a \beta-mimetic drug, ritodrine, increased the plasma 6-keto-PGF1a concentrations in women with premature uterine contractions.14 In the uterus of the guinea pig, orciprenaline effectively reduced the in vitro yield of prostaglandins E and F from myometrial tissue homogenates. 15 In preterm labor the plasma PGFM levels decreased significantly during

^{*}p < 0.05.

todrine treatment.¹⁶ The results of these in vitro and in vivo studies suggest that the stimulation of β -adrenoreceptors enhances 6-keto-PGF_{1 α} formation and uterine quiescence and that α -adrenergic stimulation increases uterine activity and PGF_{2 α} production. Which role PGE₂ plays for the balance of uterine activity is difficult to say and requires further investigation.

In this study, myometrial tissue from the late pregnant uterus was investigated. However, it is important to know whether during pregnancy myometrium strips from different weeks vary in their prostaglandin production and in their response to adrenergic stimulation. A further study is in progress and will be finished in the near future to differentiate the unstimulated and catecholamine-stimulated prostaglandin production rates of myometrium from the 28th to the 42ad week of pregnancy.

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Prevalence of type-specific group B streptococcal antibody in human sera: A study of 405 pregnant women

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Presence of immunoglobulin G antibody against the five standard serotypes of group B streptococcus was measured by means of indirect immunofluorescence in the sera of 405 women at the time of delivery in the obstetric hospital in Vancouver. Antibody to all five serotypes was present in 22% of women whereas only 9.6% had no detectable antibody to any serotype. Among 47 women with group B streptococcus vaginal colonization, IgG antibody was detected against the homologous colonizing serotype in 100%, 75%, 78%, 89%, and 100% of sera for serotypes Ia, Ib, Ic, II, and III, respectively. This contrasted with the women who had heterologous group B streptococcal vaginal colonization or no colonization in whom 71% had serum IgG antibody to serotype Ia, 36% to Ib, 51% to Ic, 66% to II, and 60% to III. Overall the serum antibody titers were low, and few women had titers greater than 1:20 for any of the five standard serotypes. (AM J OBSTET GYNECOL 1985;152:857-60.)

Key words: Group B streptococcus, maternal antibody response

Group B streptococci continue to be a major infectious cause of morbidity and mortality in the newborn infant. Studies from many countries of healthy women in their reproductive years have shown group B streptococcal vaginal colonization rates ranging from 4.6% to 26%. In North America invasive group B streptococcal disease in neonates has an average attack rate of 1.3 to 3.0 per 1000 live births for infections developing in the first week of life and an estimated incidence of 0.6 to 1.0 per 1000 live births for disease developing in the first few weeks of life. The discrepancy between the high colonization rates in mothers and the low incidence of disease in their infants is being extensively investigated by many research workers.

In 1976 Baker and Kasper² reported the first correlation of maternal antibody deficiency with susceptibility to neonatal group B streptococcal disease; further studies followed which confirmed that absence or low concentrations of type-specific group B streptococcal antibody in the sera of mothers of infants with invasive group B streptococcal disease appeared to be a contributing factor.³⁻⁵ These findings stimulated work on the development of a vaccine to provide active immunization for susceptible women.⁶ Questions remain

concerning what level of humoral immunity can be considered protective in the human neonate and what percentage of women lack protective immunity. This investigation was done to evaluate the prevalence of type-specific group B streptococcal serum IgG antibody in pregnant women admitted for delivery at an obstetric hospital in Vancouver. In addition, the incidence of group B streptococcal vaginal colonization in the patients in the study was assessed to determine what percentage of infants were at risk of developing invasive group B streptococcal disease.

Material and methods

Pregnant women admitted to the Grace Hospital, Vancouver, for delivery during four consecutive months in 1983 were recruited into the study. Grace Hospital is a 120-bed obstetric hospital, which opened in June, 1982. The patients were unselected and gave informed consent to take part in the study.

Vaginal cultures and a serum sample were obtained from patients as they were admitted to the labor and delivery suite. Four hundred and nineteen women gave consent to take part in the study; 14 patients had to be eliminated from the study because either a serum sample or a vaginal culture was not taken, which left 405 patients for evaluation.

Duplicate vaginal swabs (Marion Scientific Culturette) were collected from each study patient at the time of the first routine vaginal examination following admission of the patient for delivery. One swab was collected in the regular manner; the second swab was placed in 10 ml of selective broth medium. Four selec-

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Table I. Serotypes of 57 strains of group B streptococci

Serotype	No. of strains
Ia	7
Ib	8
Ic	9
II	9
III	14
R	3
X	2
III/R	5

tive media were evaluated which consisted of Todd-Hewitt broth with addition of the following different antibiotic combinations: (1) 10 mg/L of colistin, 15 mg/L of nalidixic acid, and 6 mg/L of gentamicin, 186 patients; (2) 10 mg/L of colistin, 80 patients; (3) 10 mg/L of colistin, 15 mg/L of nalidixic acid, and 0.1 mg/L of crystal violet, 94 patients; (4) 10 mg/L of colistin and 2 mg/L of gentamicin, 45 patients. The agents added to the Todd-Hewitt broth were chosen to suppress the bacteria frequently found as normal flora in the female genital tract without inhibiting the growth of group B streptococci.

All culture swabs were processed 5 to 24 hours after collection. For each patient both the conventional and selective swab were inoculated onto Islam's medium⁷ and incubated anaerobically (Gas Pak, BBL Microbiology Systems) for 48 hours at 35° C. The selective swab was returned to its original broth and incubated in air at 35° C for 24 hours, after which a second subculture was made in Islam's medium and incubated as above. Agar plates were examined after 24 and 48 hours of incubation for orange/yellow pigmented colonies, which were confirmed as group B streptococci by use of Streptex (Burroughs-Wellcome). Serotyping was done with use of the five standard typing antisera obtained from the Laboratory Center for Disease Control,

All serum samples were frozen at -70° C until processed. An indirect immunofluorescent assay developed by Vogel et al.⁸ was used to detect and quantitate type-specific serum IgG antibody against the five standard group B streptococcal serotypes. Fluorescein-labeled monospecific sheep antiserum to human IgG (Wellcome Reagents) was used at a working dilution of 1:400, which produced a 4+ fluorescence. Group B streptococcal control strains used were Ia DC 1918, Ib DC 1919, Ic DC 7916, II DC 7917, and III DC 1922 obtained from the Streptococcus Reference Laboratory of the Laboratory Center for Disease Control, Ottawa.

Bacteria were grown in Todd-Hewitt broth at 35° C to midlogarithmic phase (optical density 0.30 at 550 nm) and diluted 1:100 in 0.02 mol/L phosphate-buff-reed saline solution, pH 7.2. One drop of each of the

bacterial suspensions was placed in a well on a Tefloncoated fluorescent serology titration slide (Flow Laboratories Inc.), allowed to air dry, heat fixed, and immersed in cold acetone for 10 minutes. One drop of test serum diluted 1:5 in phosphate-buffered saline solution was added to each of the test wells, and the slide was incubated in a moisture chamber at room temperature for 30 minutes, washed with phosphatebuffered saline solution, and air dried. One drop of fluorescein-conjugated sheep antiserum to human IgG was added to each test well and the slide placed in a moisture chamber for a further 15 minutes. The slide was then given a 30-minute wash in phosphate-buffered saline solution. After air-drying, each well was covered with a drop of buffered glycerol and a coverslip and examined at a magnification of ×1000 with a Zeiss fluorescent microscope. Fluorescence was graded from 1+ to 4+, 1+ being considered to have no detectable antibody.

All sera producing a fluorescent reading of $\ge 2 +$ were retested with use of doubling dilutions of sera in phosphate-buffered saline solution from 1:10 to 1:160. The titer of antibody was defined as the highest dilution of serum that produced $\ge 2 +$ fluorescence.

Results

Four hundred and five women were evaluated in the study. Fifty-six women were found to have group B streptococcal vaginal colonization, which resulted in a carrier rate of 13.8%. One patient was colonized with two group B streptococcal serotypes, which gave a total of 57 isolates. Ten of the isolates were unidentifiable with the five standard group B streptococcal serotype antisera. These strains were sent to the Streptococcus Reference Centre, Laboratory Center for Disease Control, Ottawa, and found to belong to the provisional serotypes X and R, which are protein antigens that may appear alone or in association with serotypes II and III but seldom with serotype I. The distribution of serotypes isolated is shown in Table I.

In 13 women group B streptococci were isolated from the selective broth only, thus the carrier rate would have been reduced to 11.0% if conventional swabs alone had been used in the study. The number of isolates obtained from each of the four selective broths and the conventional swabs is shown in Table II; the broth of colistin and gentamicin appears superior with a group B streptococcal yield of 20%, but in a statistical comparison with the other three broths there is no significant difference (p = 0.10).

The 405 serum samples available for study consisted of 349 sera from noncolonized women and 56 sera from women with group B streptococcal vaginal colonization. Thirty-nine women had no detectable IgG antibodies to any of the five serotypes tested, giving an incidence

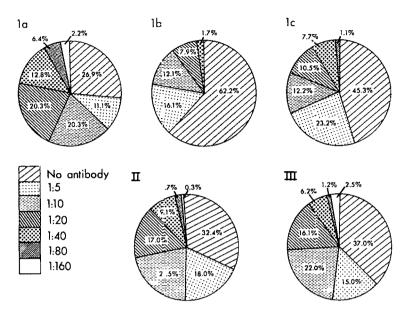


Fig. 1. Group B streptococcal IgG antibody titers in 405 pregnant women.

Table II. Comparison of group B streptococci isolates from selective broth

	Nf	gro	rts with zup B ₹ococci	No. of group B	No. of group B streptococci obtained from both plain swab
Selective broth	No. of streptococci obtained from patients No. % selective broth only	and selective broth			
Colistin, nalidixic acid, and gentamicin	186	30	16.1	6	24
Colistin	80	10	12.5	1	9
Colistin, nalidixic acid, and crystal violet	94	7	7.5	2	5
Colistin and gentamicin	45	9	20.0	4	5
Total	405	56	13.8	13	43

of 9.6%. All of these sera were from noncolonized women, and this finding was statistically significant when compared with that of the colonized women (p = 0.017).

Twenty-two percent of the patients had IgG antibodies to all five group B streptococcal serotypes tested; the incidence for colonized women was 32.1% and for noncolonized women 20.3%, which was a significant difference (p = 0.05).

When the women without group B streptococcal vaginal colonization and the women colonized with heterologous group B streptococci were combined as a group, 71% had serum IgG antibody to serotyp∈ Ia, 36% to Ib, 51% to Ic, 66% to II, and 60% to III. Among the 47 women colonized with one of the five standard group B streptococcal serotypes, IgG antibody was detected against the homologous colonizing serotype in 100%, 75%, 78%, 89%, and 100% of sera for serotypes Ia, Ib, Ic, II, and III respectively. These differences in antibody prevalence between the two groups of women were significant for serotype Ia (p = 0.001), serotype Ib (p = 0.003), serotype Ic (p = 0.000), and serotype III (p = 0.027) but not for serotype II (p = 0.299). These findings indicate that, with the exception of serotype II, vaginal colonization frequently induces the production of homologous serum antibody.

The prevalence of group B streptococcal type-specific IgG antibodies at the various titers tested in the 405 study women is shown in Fig. 1. Only a small percentage of women had serum IgG antibody titers greater than 1:20 for any of the five serotypes. The absence of any detectable antibody was high for each serotype, varying from 26.9% to 62.2% of the 405 sera tested.

None of the infants born to the 405 women in the study developed invasive group B streptococcal disease. However, there were two babies born at Grace Hospital during the four-month study period who developed early-onset group B streptococcal disease. In both patients there was group B streptococcal serotype concordance for the strains isolated from mother and infant. Antepartum serum samples from the mothers were retrieved from the blood bank laboratory for group B streptococcal antibody testing. One mather had no serum group B streptococcal IgG antibody present, and the other had antibody to the infecting sero-type in a titer of 1:5 only.

Comment

The purpose of this study was to evalute an unselected group of pregnant women to determine the group B streptococcal vaginal colonization rate and the prevalence of type-specific group B streptococcal serum IgG antibodies. The vaginal cultures and serum samples were purposely collected when the patients were admitted for delivery to assess what percentage of pabies were theoretically at risk for group B streptococcal invasive disease and to correlate this with the homologous group B streptococcal serotype serum IgC antibody titers in the colonized mothers.

Lancefield and Freimer⁹ and Lancefield et al.¹⁰ have shown that immunity to group B streptococci is trpe specific for the homologous capsular polysacchande antigen. The results show that the women with gcup B streptococcal vaginal colonization were more likely to have detectable homologous serum IgG antibocies present than noncolonized women or women with Leterologous group B streptococcal colonization, but here was no statistically significant difference in the anthody titers when the groups were compared. One enigma of invasive group B streptococcal disease is why it is an ost always restricted to the first 3 months of life and is uncommon at all other times. A further puzzle 3 the fact that these early months of life correspond to the period when passively acquired maternal serum IgG antibodies are at their zenith. Protective levels of typespecific group B streptococcal antibodies for the human neonate are not known; for one of the babies stacied a maternal serum IgG antibody titer of 1:5 was not protective.

It is apparent from several serologic studies^{2, 3, 1} that some colonized mothers with low titers of type-specific group B streptococcal antibodies or no antibodie. give birth to infants who remain well despite exposure to the organism. This suggests that other factors in addition to humoral immunity, such as the virulerce of the organism, the size of the infecting inoculum, and the natural host defences of the infant, are probably important in the pathogenesis of invasive group B streptococcal disease. Work done in animal models has shown that strain differences in virulence and inoculum size are important in the production of group B streptococcal disease.

9-11

An immunologic approach to the prevention of invasive group B streptococcal disease is inviting, and the use of group B streptococcal vaccines shows promise. ¹² Questions to be resolved concern the titers of serum IgG antibody for each of the various group B streptococcal serotypes that can be considered protective for the neonate and how long such protective titers, once achieved, are maintained in women.

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Cord blood glycosylated (glycated) hemoglobin: Correlation with maternal glycosylated (glycated) hemoglobin and birth weight

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Maternal and cord blood glycosylated hemoglobin levels were measured by an affinity chromatographic method in three groups: normal women (nondiabetic women who gave birth to infants that were normal for gestational age); test women (women who had no evidence of glucose intolerance with screening procedures and who gave birth to large—for—gestational age infants); and women with gestational diabetes. In all cases the level of cord blood glycosylated hemoglobin was approximately 40% less than the corresponding maternal blood levels, and no correlation could be detected between maternal and cord blood concentrations. The reference range for glycosylated hemoglobin in the normal maternal population was similar to that for nonpregnant adults. There was no significant difference in cord and maternal glycosylated hemoglobin levels among the three groups, although a slight upward trend was detected in the diabetic group. There was a lack of correlation of cord and maternal glycosylated hemoglobin with birth weight in all three groups. The implications of these findings are discussed in respect to the usefulness of cord and maternal glycosylated hemoglobin in retrospective screening for gestational diabetes. (Am J Obster Gynecol 1985;152:861-6.)

Key words: Glycosylated hemoglobin, birth weight, gestational diabetes

The effect of diabetes on pregnancy has been extensively studied. It is thought that maternal hyperglycemia passively produces fetal hyperglycemia, which induces fetal hyperinsulinemia. Functional hyperinsulinemia has been proposed to account for many of the complications observed in newborn infants of diabetic mothers such as hypoglycemia, hypocalcemia, respiratory distress syndrome, macrosomia, and congenital abnormalities of the spine and the skeletal, genitourinary, and cardiovascular systems.

The diagnosis of gestational diabetes is of prime importance since it has been shown that proper metabolic control during pregnancy can reduce the incidence of such anomalies. Gestational diabetes may remain undetected throughout pregnancy because of normal glucose tolerance tests with the only indication of such an abnormality being the delivery of a large-for-gestational age infant.

The diagnosis of glucose intolerance affects the management of subsequent pregnancies; therefore, its de-

tection is of prime importance.1 Many retrospective studies have been performed on mothers of macrosomic infants to screen for the presence of gestational diabetes. Once pregnancy has been terminated it is more difficult to document gestational diabetes by an oral glucose tolerance test since glucose metabolism may rapidly revert to normal post partum.3 Other routes of investigation have therefore been explored, including the measurement of maternal glycosylated hemoglobin, 4-7 which has been shown to reflect the level of glycemic control during the preceding 2 to 3 months.7 In general, the levels of maternal glycosylated hemoglobin have been shown to be a poor screening test for diabetes in pregnancy, with conflicting reports regarding the relationship between glycosylated hemoglobin levels and birth weight.4-7

Since maternal hyperglycemia is believed to passively induce fetal hyperglycemia, it has been thought that the measurement of the level of glycosylated hemoglobin in cord blood might be a more sensitive index of fetal exposure to glucose during pregnancy. The kinetics of glycosylation of fetal hemoglobins has been shown to be similar to that of adult hemoglobins and therefore to similarly reflect glycemic control. The affinity chromatography procedure with the use of maminophenylboronic acid, which has been shown to measure all glycosylated hemoglobin in umbilical cord blood, has made the analysis of cord blood glycosylated hemoglobin adaptable for routine laboratory use. This

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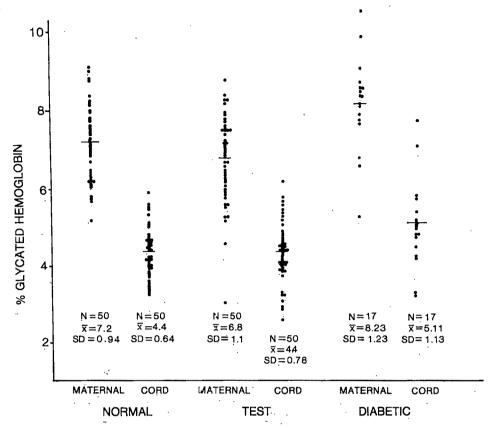


Fig. 1. Levels of maternal and cord gl=osylated (glycated) hemoglobin in normal, test, and diabetic groups.

study was designed to investigate the relation. hip of cord blood glycosylated hemoglobin to material glycosylated hemoglobin levels and birth weight.

Subjects and methods

Subjects. Maternal and cord blood was obtamed at delivery from three populations. The normal group consisted of 50 women who had no history of cabetes or other systemic diseases, who had normal valles for blood glucose screening parameters (fasting and hour postprandial blood glucose levels at 28 weeks gestation), and who gave birth at 37 to 42 weeks' getation to infants with birth weights that were appropriate for gestational age. The test population consisted of 50 women who had no history of diabetes, glucos= intolerance, or other systemic disease, who had nor al values for blood glucose screening parameters as described above, and who gave birth at 37 to 42 weeks' gestation to infants having birth weights >40\(\)0 gm. The diabetic group consisted of 17 women who were diagnosed as having gestational diabetes according to National Diabetes Data Group Criteria and wLo gave birth to infants at 35 to 40 weeks' gestational age. Women in this group did not exhibit any evidence of glucose intolerance post partum.

Methods

Collection of blood samples. Cord and maternal blood samples were collected into tubes containing ethylene-diaminetetraacetic acid at delivery. Upon reception in the laboratory, samples were centrifuged at $1200 \times g$ for 10 minutes with the plasma decanted and packed cells frozen at -20° C. Specimens have been shown to be stable for 3 months at this temperature.

Glycosylated hemoglobin analysis. Glycosylated hemoglobin in both maternal and cord blood was measured within a month subsequent to collection by an affinity chromatographic procedure with the use of m-aminophenylboronic acid in a kit purchased from Pierce Chemical Company, Rockford, Illinois, as previously described. The between-run coefficient of variation of this procedure has been shown to be <10%.

Glucose measurements. The serum glucose level was measured by a glucose oxidase/potentiometric system on an "Astra" instrument (Beckman Instruments, Brea, California) within 3 hours of procurement of the sample.

Calculation of birth weight ratios. The birth weight ratio was obtained by dividing the actual birth weight by the median (fiftieth percentile) birth weight for the particular gestational age of the infant according to published

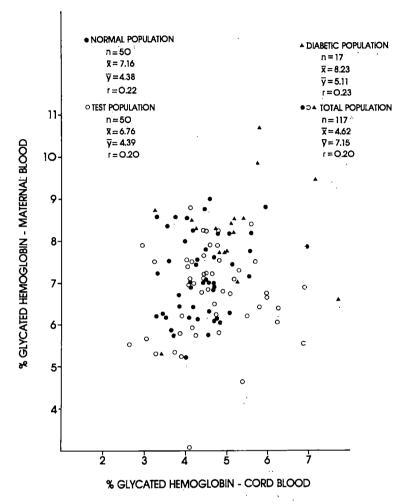


Fig. 2. Correlation of cord and maternal glycosylated (glycated) hemoglobin for the following groups: •, normal group; \circ , test group; \triangle , diabetic group.

tables.11 The approximate gestational age was based on the mother's menstrual history and ultrasound examinations.

Statistical analysis. The paired t test was used to detect differences in paired samples. The level of significance was p = 0.05. Linear regression and correlation coefficients were calculated by the method of least squares.

Results

Comparison of cord and maternal glycosylated hemoglobin. Individual and mean levels of maternal and cord blood glycosylated hemoglobin in the normal, diabetic, and test populations are shown in Fig. 1. In all cases the cord blood levels were on average 30% to 40% less than the corresponding maternal blood levels although there was some overlap in the ranges. The mean levels of cord and maternal glycosylated hemoglobin in the test and diabetic populations were not significantly higher (p > 0.05) than those of the normal group although the ciabetic group did show a trend to elevated levels. The range of both cord and maternal glycosylated hemoglobin in the normal group was in general more narrow than that seen in the other two groups. In all three populations, no correlation between the maternal and cord glycosylated hemoglobin was observed (Fig. 2).

Correlation of cord and maternal glycosylated hemoglobin with birth weight. As shown in Fig. 3, there was no significant correlation between birth weight ratios and either cord or maternal glycosylated hemoglobin levels for any of the three groups. There was very little overlap in birth weight ratios between the normal and test groups while those of the diabetic group spanned the range of both.

Comment

The levels of cord blood glycosylated hemoglobin in all three test groups were significantly lower than the corresponding maternal levels, a finding that is consistent with the reports of others.7.9.12.13 This car. be par-

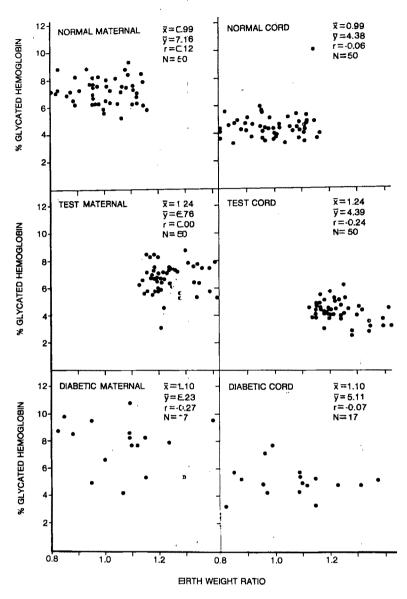


Fig. 3. Relationship of maternal and cord gly cosylated (glycated) hemoglobin to birth weight in normal, test, and diabetic groups. Birth weight ratios were determined as described in the Methods section.

tially explained by the decreased survival time of erythrocytes in the fetus^a as well as the lower levels of blood glucose to which fetal erythrocytes are exposed^a as compared to maternal cells. In addition, the glycosylation of fetal hemoglobin may be prevented in part by acetylation.⁷

Other studies have shown that glycosylated hemc-globin is either unchanged or slightly decreased of dueing normal pregnancies. The level of glycosylated hemoglobin in the maternal blood of both the normal and test groups was not significantly different from that previously reported for nonpregnant adults. This is incontrast to the previous finding with affinity chrometography that reported a decreased level of glycosylated hemoglobin in pregnant women.

The lack of correlation between maternal and cord levels of glycosylated hemoglobin in all three test groups was unexpected. Worth et al.¹² have previously shown a similar lack of correlation in diabetic subjects but a good correlation in normal subjects. In contrast, Feldman et al.⁸ have shown a good correlation between the two glycosylated hemoglobin levels in diabetic women. The reasons for these discrepancies are not known. One possibility may be that, subsequent to the initial transfer of glucose from the mother to the fetus, the latter functions as an independent unit with a hypothesized insulin response to the glucose load¹ that would tend to lead to a poor correlation between the two glycosylated hemoglobin levels. Additionally, factors other than glucose levels, as discussed above, may,

in some fetuses, have a more prominent role in determining the glycosylated hemoglobin levels.

The observation of an upward trend in both cord and maternal glycosylated hemoglobin levels in the diabetic group as compared to the normal and test groups supports the hypothesis that maternal hyperglycemia passively produces fetal hyperglycemia. The limited magnitude of this trend can be attributed to the appropriate management of these patients during pregnancy. Others have also shown similar increases in cord glycosylated hemoglobin levels in diabetic patients.8,9,12 The finding of increased C-peptide levels in cord blood of infants born to diabetic mothers14 is consistent with these observations. The limited knowledge available regarding glucose homeostasis in the fetus requires further study to determine whether elevations in the above analyses actually reflect increased fetal blood glucose levels.

The lack of correlation between birth weight ratios and cord blood glycosylated hemoglobin found in this study may be due to the fact that fetal glycosylated hemoglobin reflects only the efficiency of glucose disposal and not the amount of glucose disposed per unit of time, while lipogenesis, the primary determinant of birth weight, is a measure of the latter.1 With various methods for the quantitation of glycosylated hemoglobin, other workers have found both good8 and poor^{12, 15, 16} correlations between birth weight and cord blood glycosylated hemoglobin. A similar situation also exists for maternal glycosylated hemoglobin and birth weight.4-7 The discrepancy in results may be due in part to the different methods used for quantitation of glycosylated hemoglobin, some of which are more susceptible to interference associated with the labile and variant hemoglobin forms, one of which is hemoglobin F.7 It may also be due in part to the selection and number of patients tested, as it would be expected that the poorer the control of maternal diabetes, the better the

In a study similar to that reported here, Pollock et al.5 reported a significant increase in the level of glycosylated hemoglobin in the mothers of large-forgestational age infants as compared to appropriate control women. Since many of the former were shown to have some level of glucose intolerance, the authors suggested that glycosylated hemoglobin could possibly be used as a retrospective test for previously undetected gestational diabetes in women giving birth to largefor-gestational age infants.5 We have not been able to confirm this since we found no increase in glycosylated hemoglobin levels in maternal blood of such mothers as compared to normal control women. We have also extended this observation by showing no increase in cord glycosylated hemoglobin levels of large-for-ges-

tational infants as compared to control infants. This discrepancy may be partially due to the fact that the test population in our study exhibited normal glucose values on screening procedures, while those in the study of Pollock et al.5 did not. Therefore, because of different criteria used to establish the test group, Pollock et al.5 may have included more diabetic women, which would lead to the elevated glycosylated hemoglobin levels they observed.

The documented lack of sensitivity of glycosylated hemoglobin as a screening test for diabetes7 may be due in part to the life span of erythrocytes in the circulation. Interest has focused more recently on the clinical usefulness of glycosylated albumin and total protein as more sensitive indicators of glucose intolerance because of thei- shorter circulatory life span as compared to that of erythrocytes.7 Studies in this area are presently under vay.

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Reproductive patterns and the risk of gestational trophoblastic disease

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The relation between reproductive pattern and the risk of gestational trophoblastic disease was evaluated in a case-control study conducted in Northern Italy on 310 women with histologically confirmed gestational trophoblastic disease and two control groups consisting of 290 obstetric subjects and 394 patients in hospital for acute, nonobstetric, nongynecologic conditions. Compared to that for nulliparous women, the estimated age-adjusted relative risk of trophoblastic disease for parous women was 0.6 (90% confidence limit = 0.4 to 0.9) when obstetric controls were used as a comparison group and 0.4 (95% confidence limit = 0.2 to 0.6) compared with other controls. Conversely, a history of spontaneous abortions was associated with elevated risk of gestational trophoblastic disease, and the risk increased significantly with increasing number of spontaneous abortions. When the combined effect of parity and spontaneous abortions was considered, the major factor influencing the risk of gestational trophoblastic disease was the existence of one or more previous term pregnancies. (AM J OBSTET GYNECOL 1985;152:866-70.)

Key words: Abortion, hydatidiform mole, pregnancy, risk

There is some suggestion that gestational trophoblastic disease (hydatidiform mole and its malignant counterpart, choriocarcinoma) is more commonly associated with first pregnancy. 1.2 Other studies, however, reported a positive association between multiparity and the risk of gestational trophoblastic disease.3 These conflicting results might be explained in terms of different socioeconomic status and, of course, age distribution of cases and controls, the risk of gestational trophoblastic disease being strongly associated with age. We analyzed further the relation between reproductive pattern (parity/abortions) and benign and persistent trophoblastic disease, using data from a case-control study conducted in Lombardy, northern Italy, where the potential confounding effect of a large number of covariates could be allowed for.

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Subjects and methods

Since 1980 we have been conducting a case-control study of gestational trophoblastic disease. Trained interviewers identified and questioned cases and controls using a standard questionnaire. On the average, <2% of the eligible women (cases or controls) refused to be interviewed.

Among various facts regarding socioeconomic characteristics, personal habits, and related medical history, a detailed obstetric and reproductive history was elicited.

Cases. The cases analyzed in the present report (n = 310) were women with histologically confirmed gestational trophoblastic disease diagnosed in 10 regional hospitals in Lombardy and referred to the trophoblastic disease centers of the First and Third Obstetrics and Gynecology Clinics of the University of Milan for routine follow-up by gonadotropin assay. Pathologic examination was first performed in each separate hospital and based on at least five different blocks of tissue taken from selected areas; at least four

Table I. Distribution of 310 cases of gestational trophoblastic disease (275 benign hydatidiform mole and 35 persistent trophoblastic disease) and 290 obstetric and 394 nonobstetric control subjects according to histopathologic subgroups, number of live births, and number of abortions, Milan, Italy, 1980 to 1983

			Przor live birth	s or abortions		
	0		1		≥2	
	n	%	n	%	71	%
Live births						
Partial hydatidiform mole	16	62	7	27	3	12
Completé hydatidiform mole	137	55	73	29	39	16
Persistent trophoblastic disease	18	, 51	9	26	8	23
Obstetric controls	124	43	107	37	59	20
Other controls	87	22	95	24	212	54
Abortions					•	
Partial hydatidiform mole	19	73	6	23	1	4
Complete hydatidiform mole	192	77	41	16	16	6
Persistent trophoblastic disease	27	77	6	17	2	6
Obstetric controls	240	83	41	14	9	3
Other controls	332	84	50	13	12	3

Table II. Relative risk estimates* of gestational trophoblastic disease and 95% confidence intervals according to parity (based on data in Table I), Milan, Italy, 1980 to 1983

	Total		Be	eniga mole	Persistent disease		
	Relative risk	95% confidence interval	Relative risk	95% confidence interval	Relative risk	95% confidence interval	
Para 0		!†		 †		I†	
Para 1						,	
Obstetric controls	0.6	0.4-0.9	0.6	0.4-0.9	0.6	0.3-1.3	
Other controls	0.5	0.4-0.8	0.6	0.4-0.9	0.5	0.3-1.3	
Para ≥2							
Obstetric controls	0.5	0.3-1.0	0.5	0.3-1.0	0.5	0.2-1.5	
Other controls	0.2	0.1-0.3	0.2	0.1-0.3	0.1	0.1-0.4	

Tests for linear trend (adjusted for age)—benign mole vs. obstetric controls: $\chi_1^2 = 4.98$, p = 0.03; benign mole vs. other controls: = 51.99, p < 0.001; persistent disease vs. obstetric controls: $\chi_1^2 = 0.90$, p = 0.34; persistent disease vs. other controls: $\chi_1^2 = 0.90$, p = 0.34; persistent disease vs. other controls: $\chi_1^2 = 0.90$, p = 0.34; persistent disease vs. other controls: $\chi_1^2 = 0.90$, p = 0.34; persistent disease vs. other controls: $\chi_1^2 = 0.90$, p = 0.34; persistent disease vs. other controls: $\chi_1^2 = 0.90$, p = 0.34; persistent disease vs. other controls: $\chi_1^2 = 0.90$, p = 0.34; persistent disease vs. other controls: $\chi_1^2 = 0.90$, p = 0.34; persistent disease vs. other controls: $\chi_1^2 = 0.90$, p = 0.34; persistent disease vs. other controls: $\chi_1^2 = 0.90$, p = 0.34; persistent disease vs. other controls: $\chi_1^2 = 0.90$, p = 0.34; persistent disease vs. other controls: $\chi_1^2 = 0.90$, p = 0.34; persistent disease vs. other controls: $\chi_1^2 = 0.90$, p = 0.34; persistent disease vs. other controls: $\chi_1^2 = 0.90$, p = 0.34; persistent disease vs. other controls: $\chi_1^2 = 0.90$, p = 0.34; persistent disease vs. other controls: $\chi_1^2 = 0.90$, p = 0.34; persistent disease vs. other controls: $\chi_1^2 = 0.90$, p = 0.34; persistent disease vs. other controls: $\chi_1^2 = 0.90$, p = 0.34; persistent disease vs. other controls: $\chi_1^2 = 0.90$, p = 0.34; persistent disease vs. other controls: $\chi_1^2 = 0.90$, p = 0.34; persistent disease vs. other controls: $\chi_1^2 = 0.90$, p = 0.34; persistent disease vs. other controls: $\chi_1^2 = 0.90$, p = 0.34; persistent disease vs. other controls: $\chi_1^2 = 0.90$, p = 0.34; persistent disease vs. other controls: $\chi_1^2 = 0.90$, p = 0.34; persistent disease vs. other controls: $\chi_1^2 = 0.90$, p = 0.34; persistent disease vs. other controls: $\chi_1^2 = 0.90$, p = 0.34; persistent disease vs. other controls: $\chi_1^2 = 0.90$, p = 0.34; persistent disease vs. other controls: $\chi_1^2 = 0.90$, p = 0.34; persistent disease vs. other controls: $\chi_1^2 = 0.90$, p = 0.34; persistent disease vs. other controls: $\chi_1^2 = 0.90$, p = 0.34; p = 0.34; p = 0.34 14.38, p < 0.001; total vs. obstetric controls: $\chi_1^2 = 5.26$, p = 0.02; total vs. other controls: $\chi_1^2 = 55.45$, p < 0.001.

slides per block were obtained. Second, all pathologic material was centrally reviewed by the same pathologist. There were 26 patients with partial hydatidiform moles and 284 complete moles.

None of the 25 partial hydatidiform moles developed persistent trophoblastic disease. Among the 284 complete moles, 249 (88%) had spontaneous remissions (benign moles); 35 (12%) were treated for persistent trophoblastic disease, identified according to criteria similar to those of Bagshawe et al. 1: (1) a rising B-subunit of human chorionic gonadotropin value for at least two subsequent assays, (2) a persistently abnormal β-subunit value 4 to 6 months after evacuation of the mole, (3) persistent or recurrent uterine hemorrhage and persistently detectable \(\beta\)-subunit, and (4) clinical or histologic evidence of metastases with persistently high or rising β-subunit values.

Controls. Two different control groups were selected: the first consisted of women (n = 290) admitted for normal delivery to the same hospitals where the cases had been originally identified over comparable calendar periods. All women admitted for normal delivery in selected days were approached for interview. The second control group consisted of patients (n =394) below age 45 admitted for acute conditions to surgical, orthopedic, and a few other wards of university and general hospitals serving a catchment area comparable to that of the hospitals where cases had been identified. Women were not eligible if they were admitted for gynecologic, hormonal, or neoplastic diseases or had undergone hysterectomy or bilateral oophorectomy. Of this control series 31% were admitted because of traumatic conditions (mostly fractures and sprains), 26% had nontraumatic orthopedic con-

^{*}Adjusted for age.

[†]Reference category.

Table III. Relative risk estimates* of gestational pophoblastic disease and 95% confidence intervals according to number of abortions (based on data in Table I), Milan, Italy, 1980 to 1983

	Total		Be	nign mole	Persistent disease	
	Relative risk	95% confidenc∈ interval	Relative τisk '	95% confidence interval	Relative risk	95% confidence interval
Abortions 0		l†		l†		1†
Abortions 1						•
Obstetric controls	1.3	0.9-2.1	1.4	0.9-2.2	1.3	0.5 - 3.3
Other controls	1.9	1.2-3.1	2.0	1.2-3.3	1.3	0.2-10.8
Abortions ≥2						
Obstetric controls	2.2	1.0-5.0	2.5	1.1-5.7	1.6	0.3-7.8
Other controls	3.9	1.7-9.8	4.5	1.9-10.5	2.4	0.6-13.9

Tests for linear trend (adjusted for age)—benign mole s. obstetric controls: $\chi_1^2 = 5.41$, p = 0.02; benign mole vs. other controls: $\chi_1^2 = 18.15$, p < 0.001; persistent disease vs. obstetric controls: $\chi_1^2 = 0.50$, p = 0.48; persistent disease vs. other controls: $\chi_1^2 = 2.97$, p = 0.08; total vs. obstetric controls: $\chi_1^2 = 4.99$, p = 0.00; total vs. other controls: $\chi_1^2 = 16.79$, p < 0.001.

ditions (mostly low back pain and disk disorders), 52% had acute surgical diseases (mostly abdominal, such as acute appendicitis or strangulated hernia), and 51% had other illnesses, such as eye, ear, nose and throat disorders. The present paper is based on information collected up to December 31, 1983.

Data analysis. Odds ratios of benign, persistent and total trophoblastic disease (as estimators of relative risks)5 together with their 95% approximate conficence intervals6 were computed (separately with reference to the two control groups) according to parity and hisory of spontaneous abortions. The significance of Laear trends in risk, where appropriate, was assessed wittuse of the test given by Mantel.7 The potential reciprocal confounding effect of parity and history of spintaneous abortions was controlled for by means of aratification and the Mantel-Haenszel procedure.8 AII relative risk estimates were adjusted, in turn, for age and other potentially important covariates (number of voluntary abortions, years of education, smoking habits, ABO blood group, and age of the father). However, since adjustments for most covariates did not materially change the relative risk estimates, relative risks adj_sted for age only were chosen for presentation.

Results

The distribution of cases and controls according to histopathologic subgroups, parity, and history of scontaneous abortions is given in Table I. There we no material difference in the reproductive histories of partial or complete hydatidiform moles. Therefore the two histopathologic subcategories are presented together in the analysis. Compared with nulliparous women the estimated age-adjusted relative risk of gestational trophoblastic disease for parous women was 0.6 (95% confidence limit = 0.4 to 0.9) when obstetric controls were used as comparison group and 0.4 (95% confidence

limit = 0.2 to 0.6) when comparison was made with other controls. The protective effect of parity increased with increasing number of live births, producing statistically significant linear trends with reference to both control groups (Table II). Conversely a history of spontaneous abortions was more common in cases than in controls. The risk increased with increasing number of abortions, and the linear trend in risk was statistically significant (Table III).

Parity and number of spontaneous abortions were compared within strata of age, clinical entities (benign/persistent), education, voluntary abortions (which were reported by the same proportion of cases and controls, 7%), and other identified covariates. There was no evidence that the associations were confined to any particular subgroup. Likewise, the relative risk did not change appreciably after adjustment for these covariates by means of the Mantel-Haenszel procedure.

The interaction between parity and history of spontaneous abortions on the risk of gestational trophoblastic disease is considered in Table IV. The distribution of cases and controls according to reproductive experience was statistically heterogeneous. When women who had never been pregnant were chosen as the reference category, the risk estimates were below unity for parous women who had experienced one or more abortions, and greatly reduced in parous women with no history of spontaneous abortions. The elevated risk was apparently confined to nulliparous women with a positive history of spontaneous abortions (Table IV).

Comment

The findings of the present study suggest that the risk of gestational trophoblastic disease is significantly reduced in women with one or more previous live births. Further, in these data there was a statistically

^{*}Adjusted for age.

[†]Reference category.

significant positive association between number of spontaneous abortions and trophoblastic lesions.

The choice of two control groups seemed the most appropriate to guarantee against different potential selection biases. The obstetric control group consisted of women delivered of normal babies: this choice obviously excluded infertile controls. However, in this case, women with only abortive pregnancies also were excluded. The control group consisting of patients admitted for acute conditions may well have included some infertile women who, of course, cannot develop trophoblastic disease. Thus, although both control groups can be criticized on different grounds (that is, exclusion of fertile women with only abortive pregnancies and inclusion of infertile women) the similar results obtained give obvious evidence that the association between parity, number of spontaneous abortions, and gestational trophoblastic disease cannot easily be accounted for by selection bias.

Recall bias might have influenced the risk estimates of spontaneous abortions, cases being clearly more sensitized towards obstetric problems. However, it is obviously unlikely that recall bias might have had any effect on the reported number of live births. Further, reproductive history was just one of a large number of items on which information was elicited, and at the time of data collection, the possible relation between parity, fetal wastage, and gestational trophoblastic disease was unknown to us and to the interviewers. With regard to confounding, the results were virtually unmodified when several potential distorting factors were taken into account, including indicators of socioeconomic status and number of voluntary abortions.

It is known that partial and complete hydatidiform moles are two pathogenetically and perhaps etiologically different entities.9 In the present study, however, no matérial difference in reproductive patterns emerged between the two histopathologic subcategories, though the numbers of partial moles were possibly too limited to permit any definite conclusion.

The interpretation of previous studies on this subject is not obvious, but the results can generally be considered in agreement with the present ones. Scott' showed in a comparison with live births in the United States that choriocarcinoma was more common in association with first pregnancies. Llewellyn-Jones,3 however, reported from a high-incidence area (Malaysia) a higher risk of hydatidiform mole in multiparous (>6 births) women. Yen and MacMahon¹⁰ reported a significantly higher proportion of abortions in the prior pregnancy of the molar patients than in controls (16.0% versus 10.2%), but their overall data on gravidity showed an increasing risk with number of pregnancies as well. Finally, Baltazar 1 reported that the risk of choriocarcinoma was grossly elevated in women with one or more

Table IF. Relative risk estimates of gestational trophob astic diseases (and 95% confidence intervals) according to reproductive experience, Mi.an, I.alv, 1980 to 1983

Reprod-ctive experience	Relative risk	95% confidence interval
Para ≥ ., abortions 0		
Obstetric controls	0.6	0.4 - 0.9
Othe- controls	0.2	0.1 - 0.3
Para ≥ ., abortions ≥ l		
Obstetric controls	0.8	0.5 - 1.4
Othe- controls	0.4	0.2-0.6
Para 0, abortions 0	1*	
Para 0, abortions ≥1		
Obstetric controls	1.9	1.0-3.6
Other controls	3.3	1.4 - 7.3

3 for heterogeneity: hydatidiform mole vs. obstetric controb: 13.86, p = 0.003; hydatidiform mole vs. other controls: 92.16, p < 0.001.

previous fetal losses. Although the marked effect of the fetzl loss in that study resulted from scoring an antecedent mole as a fetal loss, the risk appeared to increase with increasing number of previous fetal losses as well.

In conclusion, though there are a few inconsistencies between the various studies, there appears to be a consistent body of evidence on the relationship between parity, history of spontaneous abortions, and trophoblastic disease. Nonetheless, even accepting the association observed in the present and other works as real, the biologic underlying explanation is not obvious.

The increased risk observed in women with a history of abortions might be caused by the fact that part of previous abortions could have been, in fact, not diagnosed hydatidiform mole, since it is known that a previous gestational trophoblastic disease increases the risk of subsequent trophoblastic disease.12 Alternatively, a simple similarity of risk factors for gestational trophoblastic disease and spontaneous abortions could account for the observed association.

However, when parity and number of previous abortiors were considered together (Table IV), the major factor influencing the risk of gestational trophoblastic disease was the existence of one or more previous term pregnancies, since the risk estimates were below unity for women with one or more children reporting preyious spentaneous abortions. Thus, a woman's ability to celiver a child could be a marker of the absence of genetic or environmental factor(s) which would favor the development of gestational trophoblastic disease.

The present study further confirms that reproductive patterns are not associated with an increased risk of persistent disease compared to benign hydatidiform mole,18,14 thus producing no important prognostic implication.

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Theoretical effects of amniotic fluid volume changes on surfactant concentration measurements

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A baseline model for consideration of the theoretical effects of amniotic fluid volume changes on surfactant concentration measurements has been constructed. The baseline data include: (1) a polynomial equation that best fits experimental data comparing amniotic fluid lecithin concentration measurements to gestational age, (2) inflow of 0.450 L of surfactant-poor fluid into the amniotic fluid per day, (3) outflow of 0.450 L of surfactant-rich fluid per day, and (4) volume of 1.0 L of amniotic fluid between 30 to 31 and 38 to 39 weeks' gestation. With this model the incremental amounts of lecithin necessary to achieve the concentration levels derived from the polynomial equation have been calculated. The quantity of lecithin needed was calculated at intervals of 0.1 day for 56 days. Polyhydramnios and oligohydramnios were then allowed to develop hypothetically by modifying the inflow or outflow of fluid while keeping the quantity of lecithin added exactly the same as was determined from the baseline data. Chronic polyhydramnios or oligohydramnios had minimal effects on the concentration measurements. The effects of acute polyhydramnios (or oligohydramnios) were greater when the change in volume was due to an increased (or decreased) volume of inflow as compared to a decreased (or increased) volume of outflow. (Am J OBSTET GYNECOL 1985;152:870-78.)

Key words: Amniotic fluid volume, surfactant concentration measurements, amniotic fluid lecithin

Considerable controversy and misconception exist regarding the relationship between amniotic fluid

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volume and surfactant concentration measurements. Some well-known researchers¹⁻³ in the field of perinatal medicine have suggested that in the face of amniotic fluid volume changes concentration measurements are subject to significant variation because of dilutional factors. Likewise, Cruz has stated unequivocally that the use of a concentration measurement rather than a ratio is subject to "great error" in conditions with associated polyhydramnios, such as diabetes mellitus. This concept is based on the belief that polyhydramnios devel-

Table I. Amount of surfactant (lecithin) necessary to achieve desired concentration measurements

Day	Lecithin added (expressed as lecithin phosphorus) (mg)
7	0.1508
14	0.2177
21	0.3039
28	0.4094
35	0.5342
42	0.6782
49	0.8416
56	1.0242

ops in much the same way as if we added a large quantity of distilled water to the amniotic fluid, thereby reducing the solute concentrations proportionately. In fact, a similar concept was in part the basis for the development of the lecithin/sphingomyelin ratio and the need to have sphingomyelin in the equation to serve as an "internal standard" to which the lecithin could be compared.⁵

At the present time a quantitative lecithin or palmitic acid measurement, the phosphatidylglycerol determination (Amniostat-FLM), the fluorescent polarization test, the button test, and the shake test, with its newer version, the Lumidex-FSI, all rely principally on the concentration of surfactant in the amniotic fluid. As newer tests are developed, it would appear very likely that the basis for these tests will be the concentration of surfactant in the amniotic fluid and the use of an "internal standard" such as sphingomyelin will gradually fade from the scene in perinatal medicine.

The purpose of this report is to present, from a theoretical and mathematical standpoint, the precise relationship between amniotic fluid volume and surfactant concentration measurements. More specifically, we examined the effect of the development of chronic and acute polyhydramnios and oligohydramnios on the amniotic fluid lecithin concentration measurement.

Methods

Baseline model. The concentration of amniotic fluid acetone-precipitable lecithin (expressed as lecithin phosphorus) has been shown to increase with respect to gestational age between 30 to 31 and 38 to 39 weeks' gestation. These data serve as the baseline surfactant concentration measurements and 30 to 31 weeks' gestation is represented by day 0 and 38 to 39 weeks by day 56 on the subsequent tables and figures. Baseline amniotic fluid volume is considered to be 1.0 L between 30 to 31 and 38 to 39 weeks and this is reasonably close to the data reported by Queenan et al. The average fetus is considered to swallow 0.450 L of surfactant-

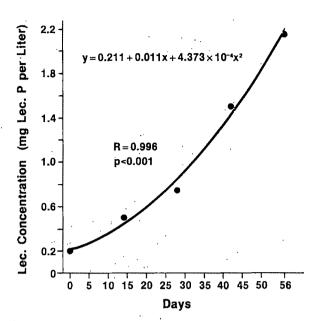


Fig. 1. Re-ationship between gestational age and amniotic fluid lecithin concentration. Baseline model = 1.0 L constant volume. Inflow, 0.450 L/day; outflow, 0.450 L/day.

rich fluid per day as reported by Pritchard.⁸ While it is fully realized that the turnover rate of water between the amniotic fluid and the maternal and fetal compartments is large, for practical purposes it is logical to consider that there is a net inflow of surfactant-poor fluid (unination) which is balanced by the outflow of surfactant-rich fluid (deglutition).

Therefore, the baseline physiologic amniotic fluid events are considered to be as follows: (1) addition of 0.450 L of surfactant-poor fluid (urination) per day, (2) removal of 0.450 L of surfactant-rich fluid (deglutition) per day (3) addition of surfactant (fetal breathing activity), and (4) constant volume of 1.0 L between 30 to 31 and 38 to 39 weeks' gestation.

Mathematical and statistical methodology. A second-degree polynominal equation was obtained by a computer regression program⁹ to describe the baseline concentration as a function of time from day 0 to day 56. This fit had a multiple correlation coefficient r value¹⁰ of 0.996 and a p value of <0.001.

The system equation describing the concentration of solute (lecithin) in the baseline model was derived as:

$$C(t) = \frac{M(t)}{V(t)} = \frac{M(t-1) + X(t) - S/V(t) \times M(t)}{V(t-1) + W - S}$$

where $C(t) = A_o + A_1t + A_2t^2$ or concentration of solute at time t; M(t) = total mass of solute at time t; M(t-1) = mass of solute at time t-1 (that is, previous increment); X(t) = incremental mass of solute added at time t; $S/V(t) \times M(t) = incremental$ mass of solute removed from solution at time t; V(t) = total volume at time t; V(t-1) = total volume at time t-1 (that is, previous

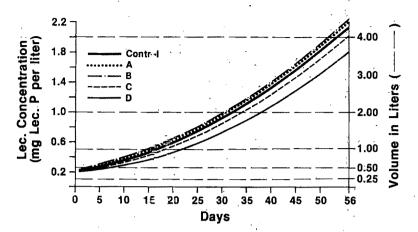


Fig. 2. Effect of maintenance of constant volume of 0.25, 0.50, 2.0, or 4.0 L as compared to baseline model of 1.0 L. Inflow, 0.450 L/day; purflow, 0.450 L/day. A = 0.50 L; B = 0.25 L; C = 2.00 L; D = 4.00 L.

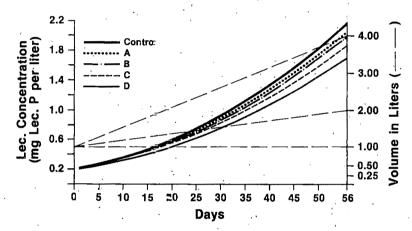


Fig. 3. Effect of development of chrome polyhydramnios. A: Inflow, 0.450 L/day; outflow, 0.432 L/day; final volume, 2.00 L. B: Inflow, 0.458 L/day; outflow, 0.450 L/day; final volume, 2.00 L. C: Inflow, 0.450 L/day; outflow, 0.396 L day; final volume, 4.00 L. D: Inflow, 0.504 L/day; outflow, 0.450 L/day; final volume, 4.00 L.

increment; W = incremental volume of solvent: dded; S = incremental volume of solution removed.

The first derivative with respect to time of the polynomial equation was set equal to the differential of the system equation and was solved for the rate of accilizion of solute to the model volume as shown: $X(t) = \sqrt{t} \times C(t)' + C(t) \times W$, where $C(t)' = A_1 + 2A_2t$. The system equation was then used in a computer program to calculate the concentration of solute under varying volumetric conditions while the rate of solute addition was held constant. A value of 10 calculative increments per day was chosen and proved sufficient to yield negligible errors, that is, when the baseline flow condition were set into the program and the results compared to the derived concentration equation data, there was greement of better than 99.95% on any day.

The computer model allows a slight incremental swelling of the volume for calculative purposes Each increment consists of three operations, which are: (1)

addition of an incremental amount of solute and solvent; (2) assuming instantaneous mixing, calculation of the concentration of the slightly enlarged volume of solution; (3) withdrawal of an incremental amount of solution from the volume.

A total of 560 increments was performed and the results were automatically plotted, while 56 daily values were printed. The computer program enables the user to change the rate of solvent input, the rate of solution removal, and the initial volume of the model. Therefore, chronic or acute polyhydramnios or oligohydramnios can be simulated.

Results

With the use of data previously reported,⁶ a curve of best fit was determined and is illustrated in Fig. 1. It is clearly evident from the r and p values that this second-degree polynomial equation fits the experimental data very closely.

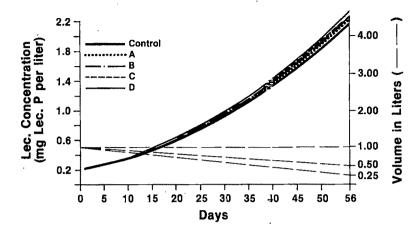


Fig. 4. Effect of development of chronic oligohydramnios. €: Inflow, 0.450 L/day; outflow, 0.459 L/day; final volume, 0.50 L. B: Inflow, 0.450 L/day; outflow, 0.463 L/day; final volume, 0.25 L. C: Inflow, 0.441 L/day; outflow, 0.450 L/day; final volume, 0.50 L. D: Inflow, 0.437 L/day; outflow, 0.450 L/day; final volume, 0.25 L.

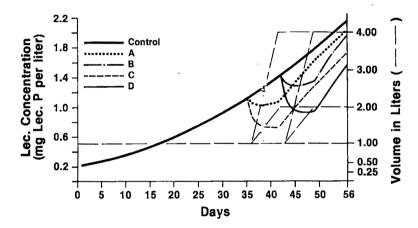


Fig. 5. Effect of development of acute polyhydramnios, increased inflow, 1 week. A: Inflow, 0.593 L/day; outflow, 0.450 L/day; days 35 to 42; final volume, 5 00 L. B: Inflow, 0.593 L/day; outflow, 0.450 L/day; days 42 to 49; final volume, 2.00 L. C: Inflow, 1.878 L/day; outflow, 0.450 L/day; days 35 to 42; final volume, 4.00 L. D: Inflow, 0.878 L/day; outflow, 0.450 L/day; days 42 to 47; final volume, 4.00 L.

With the hypothesis that 0.450 L of surfactant-poor fluid is added to and 0.450 L of surfactant-rich fluid is removed from the amniotic cavity each day, leaving a constant volume of 1.0 L, the amount of surfactant (lecithin) necessary to achieve the concentration values in Fig. 1 was calculated. While the actual calculations were made so that lecithin was added to the solution 10 times per day, Table I was constructed (as an example) to illustrate the amount added per day on each of 8 specific days. Once the amount of lecithin necessary to be added incrementally every 0.1 day for 56 days was determined, these data were kept consistent throughout the remainder of the study design.

Fig. 2 illustrates the effect of maintaining a constant amniotic fluid volume of 0.25, 0.5, 2.0, or 4.0 L from day 0 to day 56 as compared to the baseline value of 1.0 L. The concentration measurements made on day

56 with 0.25 and 0.5 L of amniotic fluid were 4.6% and 3.0% higher than the baseline value and the measurements made with 2.0 and 4.0 L of amniotic fluid were 5.5% and 15.6% lower than baseline.

One can induce an increase in amniotic fluid volume by increasing the inflow and keeping the outflow constant or by decreasing the outflow and keeping the inflow constant. Opposite changes will induce a decrease in volume. For the purpose of this report chronic polyhydramnios (or oligohydramnios) was defined as a twofold or fourfold increase (or decrease) in volume of 1.0 L during a time period of 56 days. Acute polyhydramnios (or oligohydramnios) was defined as similar changes over a time period of 7 or 14 days.

Fig. 3 simulates the development of chronic polyhydramnios by increasing the inflow (B and D) while keeping the outflow constant and by keeping a constant

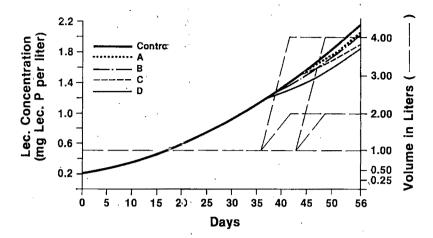


Fig. 6. Effect of development of acute polyhydramnios, decreased outflow, 1 week. A. Inflow, 0.450 L/day; outflow, 0.307 L/day; days 42 to 49; final volume, 2.00 L. B. Inflow, 0.450 L/day; outflow, 0.307 L/day; days 35 to 42; final volume, 2.00 L. C. Inflow, 0.450 L/day; outflow, 0.021 L/day; days 42 to 49; final volume, 4.00 L. D. Inflow, 0.450 L/day; outflow, 0.021 L/day; days 35 to 42; final volume, 4.00 L.

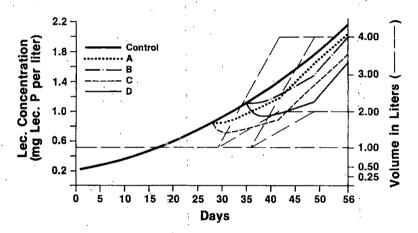


Fig. 7. Effect of development of acute pdyLydramnios, increased inflow, 2 weeks. A: Inflow, 0.522 L/day; outflow, 0.450 L/day; days 28 to -2; final volume, 2.00 L. B: Inflow, 0.522 L/day; outflow, 0.450 L/day; days 35 to 49; final volume, 2.00 L. C: Inflow, 0.664 L/day; outflow, 0.450 L/day; days 28 to 42; final volume, 4.00 L. D: Inflow, C664 L/day; outflow, 0.450 L/day; days 35 to 49; final volume, 4.00 L.

inflow (A and C) while decreasing the outflow. These changes resulted in an increase in amniotic fluid volume from 1.0 to 2.0 L (B and A) or 4.0 L (D and C), while the concentration was decreased at day 56 by 8.3% (E), 21.7% (D), 5.1% (A), and 14.3% (C).

The development of chronic oligohydramnios $s \equiv 1$ -lustrated in Fig. 4. In the first instance the outflow was kept constant (C and D) while the inflow was reduced. In the second instance the inflow was kept constant (C and C) while the outflow was increased. In these cases the volume was reduced from 1.0 to 0.5 L (C and C) or 0.25 L (C and C). The concentration measurem C made on day 56 were 5.1% (C), 7.8% (C), 3.2% (C), and 4.6% (C) higher than baseline.

The effects of acute polyhydramnios are shown in Figs. 5 through 8. Fig. 5 represents an increase in indov

for I week with a constant outflow. The graph also demonstrates the effect of variation of time of development of polyhydramnios, that is, day 35 to day 42 or day 42 to day 49. The percentage decrease in the concentration measurements made on day 56 compared to baseline was as follows: days 35 to 42, 6.0% (A) and 19.8% (C); days 42 to 49, 9.1% (B) and 27.2% (D). If the mechanism of development of acute polyhydramnios was different, that is, a constant inflow and a reduced outflow for 1 week, then the results were considerably altered as illustrated in Fig. 6. The concentration measurements on day 56 compared to baseline were decreased as follows: days 35 to 42, 5.5% (B) and 12.0% (D); days 42 to 49, 5.1% (A) and 8.1% (C). Also the patterns of the curves were strikingly different. The immediate effect of acute polyhydramnios was

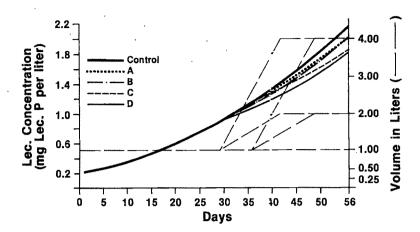


Fig. 8. Effect of development of acute polyhydramnios, decreased outflow, 2 weeks. A: Inflow, 0.450 L/day; outflow, 0.379 L/day; days 35 to 49; final volume, 2.50 L. B: Inflow, 0.450 L/day; outflow, 0.379 L/day; days 28 to 42; final volume, 2.00 L. C. Inflow, 0 450 L/day; outflow, 0.236 L/day; days 35 to 49; final volume, 4.00 L. D. Inflow, 0.450 L/day; outflow, 0.236 L/day; days 28 to 42; final volume, 4.00 L.

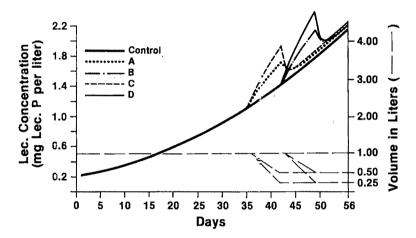


Fig. 9. Effect of development of acute oligohydramnios, decreased inflow, I week. A: Inflow, 0.378 L/day; outflow, 0.450 L/day; days 35 to 42; final volume, 0.50 L. B. Inflow, 0378 L/day; outflow, 0.450 L/day; days 42 to 49; final volume, 0.50 L. C. Inflow 0.343 L/day; outflow, 0.450 L/day; days 35 to 42; final volume, 0.25 L. D: Inflow, 0.343 L/day; ou flow, 0.450 L/day; days 42 to 49; final volume, 0.25 L.

much greater when the polyhydramnios was due to increased inflow (Fig. 5) than when it was due to decreased outflow (Fig. 6).

Figs. 7 and 8 illustrate similar changes when the polyhydramnios developed during a period of 2 weeks. The concentration measurements made on day 56 in Fig. 7 were reduced by 6.0% (A), 18.4% (C), 7.4% (B), and 23.0% (D). The reductions shown in Fig. 8 were 5.5% (A), 13.4% (C), 5.5% (B), and 14.7% (D).

The results of the development of acute oligohydramnios are presented in Figs. 9 to 12. Fig. 9 illustrates the effect of a reduction of inflow and a constant outflow for a period of 1 week. The effects of these changes on concentration measurements made on day 56 were as follows: days 35 to 42, +3.2% (A) and +4.6% (C); days 42 to 49, +3.2% (B) and +4.6% (D). Fig. 10 demonstrates the maintenance of a constant inflow and an increase in outflow during a 1-week period. The effects on the concentration measurements made on day 56 were as follows: days 35 to 42, +3.2% (C) and +4.6%(D); days 42 to 49, +3.2% (A) and +4.6% (B).

While there was no difference in the measurements made on day 56 between Figs. 9 and 10, the patterns of the curves again were markedly different. The immediate effect of acute oligohydramnios was more when the oligohydramnios was the result of reduced inflor (Fig. 9) than when it resulted from increased outflew (Fig. 10).

The effects of the development of acute oligohydramnios during a period of 2 weeks are illustrated in Figs. 11 and 12. The concentration measurements made on day 56 in Fig. 11 were increased by 3.2% (A),

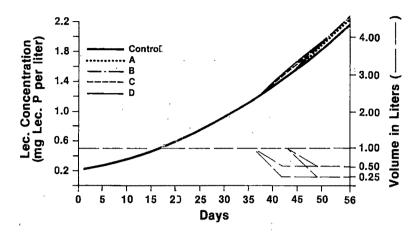


Fig. 10. Effect of development of acute * ligchydramnios, increased outflow, 1 week. A: Inflow, 0.450 L/day; outflow, 0.522 L/day; days 42 to 49; final volume, 0.50 L. B: Inflow, 0.450 L/day; outflow, 0.557 L/day; days 42 to 49; final volume, C.25 L. C: Inflow, 0.450 L/day; outflow, 0.522 L/day; days 35 to 42; final volume, 0.50 L. D: Inflow, 0.450 L/day; outflow, 0.557 L/day; days 35 to 42; final volume, 0.25 L.

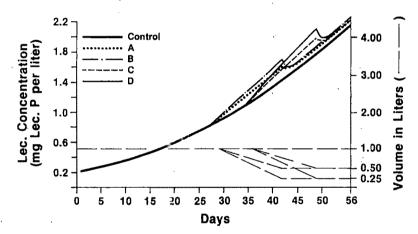


Fig. 11. Effect of development of acute of gchydramnios, decreased inflow, 2 weeks. A: Inflow, 0.414 L/day; outflow, 0.450 L/day; days 28 to 42; final volume, 0.50 L. B: Inflow 0.397 L/day; outflow, 0.450 L/day; days 28 to 42; final volume, 0.25 L. C: Inflow, 0.414 L/day; outflow, 0.450 L/day; days 35 to 49; final volume, 0.50 L. D: Inflow 0.397 L/day; outflow 0.450 L/day; days 35 to 49; final volume, 0.25 L.

4.6% (B), 3.2% (C), and 4.6% (D). In Fig. 12 the increases shown were 3.2% (B), 4.6% (D), 3.2% (A), and 4.6% (C).

Comment

It is quite clear from Fig. 2 that the maintenance of a constant volume of amniotic fluid between 0.25 and 4.0 L between 30 to 31 and 38 to 39 weeks' gestation has relatively little effect on the concentration measurement compared to the 1.0 L constant volume. The largest effect was seen as a 15.6% dilutional effect with a 4.0 L volume.

Likewise, the development of chronic polyhydrannios (gradual increase in volume from 1.0 to 2.0 $_$ or 4.0 L) or chronic oligohydramnios (gradual decrease in volume from 1.0 to 0.5 L or 0.25 L) had a slight but

small effect on the concentration measurement. The greatest effect was seen as a 21.7% dilutional effect when the polyhydramnios (300% increase in volume) was brought about by an increase in inflow. There was a lesser effect when the polyhydramnios was caused by a decreased outflow. In chronic oligohydramnios caused by a decreased inflow there was only a 7.8% increase in concentration when there was a 75% reduction in volume (1.0 to 0.25 L). There was a lesser effect when the oligohydramnios was the result of an increased outflow.

The greatest effect of acute polyhydramnios (Figs. 5 and 7) was seen when the development of the polyhydramnios (increase in volume of 300%) occurred during a period of 1 week just preceding the sampling of the amniotic fluid and was the result of an increase

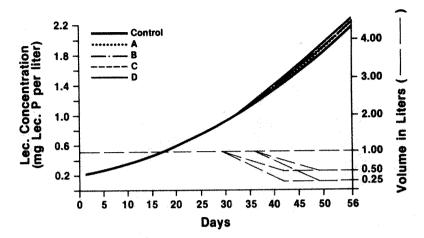


Fig. 12. Effect of development of acute oligohydramnios, increased outflow, 2 weeks. A: Inflow, 0.450 L/day; outflow, 0.486 L/day; days 35 to 49; final volume, 0.50 L. B: Inflow, 0.450 L/day; outflow, 0.486 L/day; days 28 to 42; final volume, 0.50 L. C. Inflow, 0.450 L/day; outflow, 0.504 L/day; days 35 to 49; final volume, 0.25 L. D: Inflow, 0.450 L/day; outflow, 0.504 L/day; days 28 to 42; final volume, 0.25 L.

in volume of inflow as compared to a reduction in volume of outflow. As can be seen in Figs. 6 and 8, some acute defect in fetal swallowing (outflow) resulting in polyhydramnios would have much less effect on the concentration measurement.

Likewise, the greatest effect of acute oligohydramnios was seen when the oligohydramnios occurred just prior to amniotic fluid sampling and was caused by a decrease in volume of inflow (Figs. 9 and 11) and not an increase in volume of outflow (Figs. 10 and 12). Therefore, some acute defect in fetal urination (inflow) might significantly affect the concentration measurement if the timing of the sample was such that the amniocentesis was done just at the end of the maximum decrease in volume. If additional surfactant-poor fluid were to begin to enter the amniotic cavity at the end of the acute oligohydramnios event, thereby stabilizing the volume at some lower level, the effect of acute oligohydramnios on the concentration measurement would be significantly diminished, as seen in Figs. 9 and 11.

Since the obstetrician would more commonly encounter idiopathic polyhydramnios or oligohydramnios, it would generally not be known if the problem were one of inflow or outflow. However, one might be expected to realize the problem as being one of a chronic or an acute nature; one might be expected to know the time the problem developed relative to the time of the amniocentesis; and one might be expected to realize in certain cases that the problem lay with fetal swallowing or urination. In those cases, an understanding of these concepts, which are summarized in Table II, might well aid the obstetrician in an intelligent evaluation of how much a condition of polyhydramnios or oligohydramnios affects the surfactant concentration

Table II. Effect of polyhydramnios or oligohydramnios on an amniotic fluid surfactant concentration measurement

Type	Effect
Polyhydramnios	
Constant (between 30-38 wk)	Small
Chronic (develops gradually)	
Increased inflow	Small
Decreased outflow Acute (develops rapidly)	Small
Increased inflow	May be significant especially if amniocentesis is done at end of development
Decreased outflow	Small
Oligohydramnios	
Constant (between 30-38 wk) Chronic (develops gradually)	Minimal
Decreased inflow	Minimal
Increased outflow	Minimal
Acute (develops rapidly)	
Decreased inflow	May be significant only if amniocentesis is done during or just after develop- ment
Increased outflow	Minimal

measurement being reported. In any event, it is quite clear that variations in amniotic fluid volume do not influence the results of surfactant concentration measurements to anywhere near the extent currently believed by some workers in the field.

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Intraligamentary pregnancy resulting in a live infant

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Presented is the case of a woman with an ectopic pregnancy in the left broad ligament, which resulted in a live, healthy infant. Despite the characteristic history of an abdominal crisis early in pregnancy and complaints of abdominal pain throughout pregnancy, the correct diagnosis was not established until cesarean section. (AM J OBSTET GYNECOL 1985;152:878-9.)

Key words: Ectopic pregnancy, intraligamentary pregnancy, abdominal pregnancy

Third-trimester abdominal pregnancy is a rare condition associated with a high fetal loss as well as with severe maternal hemorrhage. We present a case of an intraligamentary pregnancy resulting in the delivery of a live and healthy infant at 36 weeks' gestation.

The patient, a healthy 20-year-old, black woman, gravida 4, para 3, was admitted to the Department of Surgery in the twelfth week of pregnancy because of acute pain in the right iliac fossa. Following the diagnosis of acute appendicitis, a right lower quadrant laparotomy was performed. A small amount of blood was found in the abdominal cavity, but the appendix appeared to be normal. Through the small incision only the right side of the enlarged uterus and the right tube and ovary could be inspected; no gross abnormalities were seen. Because a recent ultrasonogram had been interpreted as showing the presence of an intrauterine gestational sac, an ectopic pregnancy on the left side was considered unlikely. No more bleeding was observed, and the abdomen was closed. The patient was discharged and the pregnancy continued. Except for continuing complaints of abdominal pain of varying

Fig. 1. The situation after delivery and after partial removal of the placenta as seen from behind. *1*, Uterus; *2*, left broad ligament; *3*, left fallopian tube; *4*, gauze compressing the oozing placental bed.

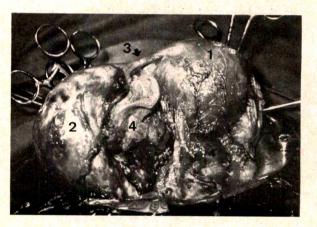
severity without any apparent cause, there were no major problems until 32 weeks. At that time she developed hypertension for which she was admitted at 34 weeks' gestation. Several ultrasound examinations were performed which showed a normal and apparently intrauterine fetus in cephalic position. Soon after admission the patient complained of severe abdominal pain, which was associated with an acute drop in hemoglobin concentration. A diagnosis of partial abruptio placentae was considered but could not be confirmed; repeat fetal

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cardiotocograms revealed no signs of fetal distress. At 36 weeks' gestation cesarean section was performed because of severe preeclampsia. During the operation the products of conception appeared to be situated between the folds of the left broad ligament. A transverse incision was made in the anterior fold of the broad ligament, and a normal male infant was delivered weighing 2560 gm with Apgar scores of 7 and 9 at 1 and 5 minutes, respectively. The placenta was attached to the left side of the uterus and to the posterior fold of the broad ligament which was covered by large vessels. Two units of packed cells were available, and it was decided to remove the placenta. The situation after partial removal of the placenta is shown in Fig. 1. Hemorrhage was controlled by pressure at the bleeding sites and by clamping and removal of the remainder of the left broad ligament with the left ovary and fallopian tube. Small parts of the placenta firmly attached to the uterus and the pelvic floor were left behind. The postoperative course was uneventful, and blood pressure returned to normal within 3 days. Two weeks after the operation the level of $\beta\text{-}\text{human}$ chorionic gonadotropin in serum was below 2 ng/ml, and it was concluded that no active placental tissue was present. Gynecologic examination after 6 weeks revealed no abnormalities.

In retrospect we assume that the patient had an ectopic pregnancy in the left fallopian tube, which in the twelfth week of gestation ruptured into the left broad ligament. The products of conception continued to de-

velop normally, but throughout pregnancy the woman complained of abdominal pain for which no cause could be found. This complaint, together with the abdominal crisis early in pregnancy, has been described as being characteristic of abdominal pregnancy.\(^1\) Nevertheless, the diagnosis was not established, which may have been the result, at least in part, of the reassuring ultrasonograms. The ultrasound image of an intrauterine pseudogestational sac may be misleading in cases of ectopic pregnancy.

An important decision concerns what to do with the placenta. Zuspan et al.¹ emphasize that no dogmatic statement regarding the management of the placenta can be made; each case must be managed individually. In our case parts of the placenta were left behind, and resorption was quick and apparently complete.

An interesting observation is the development of severe preeclampsia which, in the absence of preexisting vascular or renal disease, occurred in this multiparous woman and which has been previously reported to occur in patients with abdominal pregnancy.²

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Mondor's disease in the breast

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Mondor's disease is thrombophlebitis of a vein on the anterolateral thoracoabdominal wall. When it involves the breast it may cause undue apprehension. The characteristic appearance is described and illustrated. A mammogram is shown. (AM J OBSTET GYNECOL 1985;152:879-81.)

Key words: Mondor's disease, breast, mammography

Mondor's disease occurs most often in females.^{1, 2} It is a rare, benign, thrombophlebitis of a superficial vein on the anterolateral thoracoabdominal wall, usually in, or near a breast. Because it commonly is seen and felt

in the breast, it may, when unrecognized, cause undue concern and biopsy.

The thrombosed vein presents acutely in the breast as a painful cordlike structure. It may be tautened when the arm is elevated. The thrombosed vein may be raised like a bowstring or lie in a groove or furrow. Occasionally, it may branch or be shaped like a V or an inverted V. Palpation of it may be better than visual observation; but when it is visible, the appearance is characteristic, and the breast furrows seen in our patients bore a striking resemblance to each other (Figs. 1, A, and 2), and to examples in the literature.

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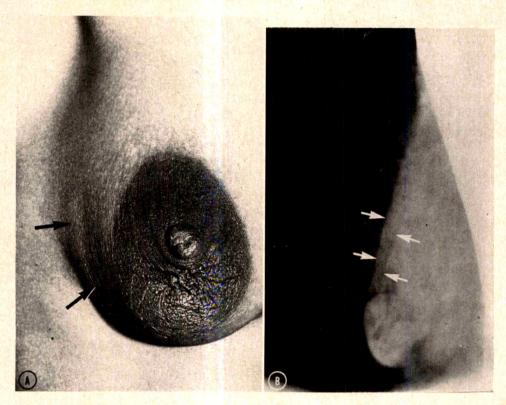


Fig. 1. A, Classical long furrow seen with Mondor's disease in the breast. B, Mammogram taken in oblique projection demonstrating elongated, sharply demarcated shadow coinciding with cordlike thrombosed vein felt on palpation. Skinfold may contribute to shadow.

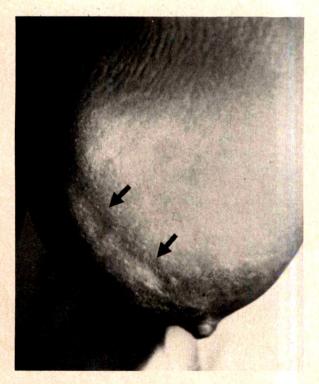


Fig. 2. Another typical example of Mondor's disease. (Reproduced with permission from Camiel MR, Benninghoff DL. Mondor's disease in the breast—superficial thrombophlebitis of the thoracoabdominal wall. J Natl Med Assoc 1971;63: 352-3.)

Case report

A 23-year-old woman was seen with an 8-hour history of having awakened with severe pain and tenderness in her right breast. By afternoon, when she was first examined, much of the pain had subsided. A 5.0 cm long, tender, cordlike structure was palpated on the outer side of the breast, with extension toward the outer lower edge of the areola. When the arm was raised, a typical furrow could be seen. The tender cord was diagnosed as a thrombosed vein, and the condition was recognized as Mondor's disease (Fig. 1, A). No oral contraceptives had been taken during the prior 8 months. On mammography, the thickened, thrombosed vein and associated furrow could be identified (Fig. 1, B). Discomfort continued for about 36 hours and thereafter was intermittent for about a week, after which no signs or symptoms remained.

Mondor's disease is usually described in relationship to the breast, but it may occur anywhere on the anterolateral thoracoabdominal wall. It may be seen in males. The cause is unknown. Trauma, breast biopsy, tight bandaging, and heavy pendulous breasts have been suspected. The pathologic picture is that of thrombophlebitis, with the thickened cord being due to thrombus formation and sclerosing phlebitis. The condition is unrelated to pregnancy.

The diagnosis is usually made clinically by the typical appearance and course. There is no reason to suspect

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malignancy, but mammograms taken in suitable projections should be helpful in the differential diagnosis. If malignancy is suspected by those familiar with Mondor's disease, a biopsy is indicated. Thermograms may prove to be helpful. Infrared photographs were not successful in this patient, but when well taken they may show the thrombosed vein and contiguous veins in excellent diagnostic detail.

The disease is self-limiting, usually lasting from 2 to 4 weeks. Treatment is symptomatic.

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Benign nevus (ephelis) of the uterine cervix

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A case report of a benign nevus of the cervix, consistent with an ephelis, is presented. (AM J OBSTET GYNECOL 1985;152:881-82.)

Key words: Nevus, cervix, ephelis

Although the presence of melanocytes in the uterine cervix was described over 60 years ago, pigmentation of the cervix is an unusual occurrence. Of the benign pigmented lesions that occur in the skin (i.e., ephelis, lentigo, junctional nevus, intradermal nevus, and blue

nevus), only cases of blue nevi and a single case of lentigo¹ have thus far been described as occurring in the uterine cervix. The following report describes a case of a benign nevus of the cervix, consistent with an ephelis. Biopsy of the lesion is necessary to rule out the possibility of malignant melanoma.²

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Case report

A hospitalized 48-year-old black, multiparous woman presented for a routine gynecologic evaluation. Her last such examination had been approximately 4 years pre-

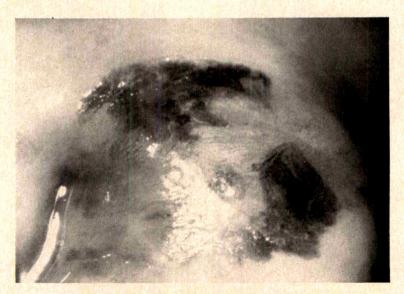


Fig. 1. Appearance of the lesion as seen with the colposcope (×13.5). Note the area of depigmentation due to the first biopsy.

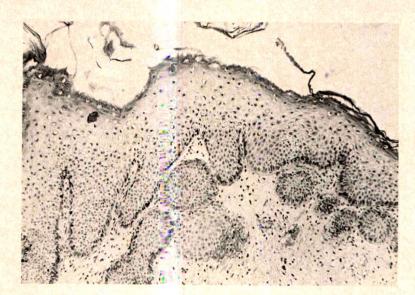


Fig. 2. Microscopic appearance (\times 165) of the lesion. Note the uniform hyperpigmentation of the basal layer and the lack of melanocytic activity.

viously. She stated that, to her knowledge, the findings of all prior pelvic examinations and Papanicolaou smears had been unremarkable. Her menstrual history was without irregularity. A postpartum tubal ligation provided her with contraception. The patient was currently under treatment for chronic obstructive pulmonary disease and cor pulmonale.

The patient was grossly obese. Pelvic examination revealed a slightly gaping introitus with a moderate rectocele formation. The cervix demonstrated a 1.5 by 1.0 cm. brownish discoloration in the periphery of the portio from 11 to 2 o'clock (Fig. 1). The lesion did not involve the external os. A Papanicolaou smear and a sample biopsy specimen were obtained, and colposcopy was performed. Colposcopic findings were unremarkable except for the pigmentation changes. The biopsy report was "nevus of the cervix." When the unusual nature of this lesion was appreciated, the patient was requested to return for colpophotographs and further histologic study. Additional biopsy material showed by-

perkeratosis, parakeratosis, and pigmentation of the basal layer. Melanocytic activity was not observed (Fig. 2). The lesion was diagnosed as ephelis of the cervix.

Pigmentation of the cervix is an unusual occurrence. Although most of the lesions described have been blue nevi or, more rarely, lentigo, 1 primary melanoma of the cervix is also a possibility. 2 Even though the cytologic smear could provide a clue as to the nature of the lesion in some cases, biopsy would still be necessary for a complete evaluation and an accurate differential diagnosis.

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Polyhydramnios and obstructive renal failure: A case report and review of the literature

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Described is a pregnancy complicated by pregnancy-induced hypertension, polyhydramnios, and obstructive renal failure due to an overdistended uterus. A review of the literature disclosed that only five such cases have been reported previously. Fetal outcome was generally related to the duration of gestation at the onset of polyhydramnios. (AM J OBSTET GYNECOL 1985;152:883-5.)

Key words: Polyhydramnios, obstructive renal failure

Although uncommon, acute renal failure in pregnancy is usually the result of decreased renal blood flow due to hypovolemia, septic shock, or severe preeclampsia/eclampsia. The purpose of this report is to describe a pregnancy complicated by twins, pregnancy-induced hypertension, polyhydramnios, and obstructive renal failure, as well as to review all reported cases¹⁻⁴ of obstructive renal failure in pregnancy caused by polyhydramnios.

Case report

A 33-year-old white woman, gravida 3, para 2, was referred to the University of Connecticut Health Center at 28 weeks' gestation for evaluation of marked polyhydramnios associated with dyspnea, orthopnea, abdominal discomfort, and threatened premature labor. On the day prior to the referral, an amniocentesis had been performed, with the withdrawal of 1100 ml of amniotic fluid. Significant history included a primary cesarean section at term in 1980 for placenta previa, and a repeat cesarean section in 1982.

On admission, the blood pressure was 140/90 mm Hg, pulse was 116 bpm, and respiratory rate was 28 per minute. The abdomen was tense and tender, and the uterine fundus measured 47 cm above the pubic symphysis. There was mild peripheral edema and hyperactive deep tendon reflexes (4+) with three beats of clonus. Regular uterine contractions were present. The cervix was 1 cm dilated and 100% effaced. Findings from the rest of the physical examination were unremarkable.

Ultrasound evaluation on admission revealed the presence of a twin gestation with discordant fetal growth and gross polyhydramnios surrounding twin A.

Abnormal laboratory findings included: blood ureanitrogen, 45 mg/dl (normal, 7 to 15 mg/dl); serum creatinine, 2.6 mg/dl (normal, 0.5 to 0.8 mg/dl); and uric acid, 13.7 mg/dl (normal, 2 to 6 mg/dl). The serum sodium was 126 mEq/L (normal, 135 to 145 mEq/L) and bicarbonate was 20 mEq/L (normal, 24 to 30). Catheterization of the bladder yielded only 35 ml of dark-colored urine. The urinalysis revealed a specific gravity of 1.017, 2+ proteinuria, 10 to 15 red blood cells per high-power field, 0 to 2 hyaline casts, and 0 to 4 granular casts per high-power field. Arterial blood gases obtained on 2 liters of oxygen mask revealed a pH of 7.39, Po₂ of 123 mm Hg, Pco₂ of 31 mm Hg, and HCO₃ of 19 mmol/L.

In spite of intravenous hydration, the urinary output remained low (5 to 8 ml per hour) and the blood pressure elevated (140 to 160/85 to 90 mm Hg). Regular uterine contractions persisted and progressive cervical dilatation was documented. The patient was given magnesium sulfate intravenously and underwent a repeat cesarean section approximately 6 hours after admission. At the time of the cesarean section, gross polyhydramnios was noted when the sac of twin A was ruptured; this infant had Apgar scores of 7 and 7 at 1 and 5 minutes, respectively. A small amount of amniotic fluid was noted when the amniotic sac of twin B was ruptured. Twin B was delivered with Apgar scores of 7 and 8 at 1 and 5 minutes, respectively. Immediately after the uterus was emptied, marked diuresis of a large amount of clear urine (approximately 250 to 300 ml per hour) began. On the first postoperative day, the blood urea nitrogen and serum creatinine were 32 mg/dl and 1.1 mg/dl, respectively. On the second postoperative day, the blood urea nitrogen was reported to be 14 mg/dl and the serum creatinine was 0.8 mg/dl. The patient was discharged on the seventh postoperative day with normal renal function and urinalysis.

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Table I. Clinical findings in polyhydramnios associated with obstructive renal failure

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Author(s), year	Case No.	Gestational age on admission (wk)	Multiple gestation	Indication(s) for admission	On admission		
					Serum creatinine (mg/dl)	Blood urea nitrogen (mg/dl)	Uric acid (mg/dl)
Quigley and Gruikshank ¹ (1977)	1	33	Twins	Dyspnea, abdominal discomfort	3.8	47	10.6
	2	21	Twins	Dyspnea, orthopnea	Not reported	Not reported	Not reported
O'Shaughnessy et al. ² (1980)	3	34	Twins	Premature cervical di- latation	7.6	42	11.1
Hamilton et al. ³ (1980)	4	26	Twins	Bed rest	8.4	98.4	Not reported
Seeds et al. ⁴ (1984)	5	29.5	Singleton with fetal renal cyst	Hypertension, hydramnios	1.9	12	Not reported
Vintzileos et al. (previously unreported case)	6	28	Twins	Dyspnea, abdominal discomfort	2.6	45	13.7

Table II. Treatment and outcome in polyhydramnios associated with obstructive renal failure

Case No.	Treatment	Pregnancy prolongation (days)	Fetal outcome	Renal function returned to normal (days) after delivery
1	Amniocentesis (1900 ml), amniotomy, delivery	3	Twin A, 2000 gm Twin B, 1330 gm	<4
2	Amniocentesis (2000 ml) amniotomy, delivery	3	Twin A, 475 gm (stillborn) Twin B, 435 gm (stillborn)	Not reported
3	Knee-chest position, amnio- centesis (1000 ml), amni- otomy, delivery	3	Twin A, 1980 gm Twin B, 1860 gm	4
4	Transabdominal drainage of amniotic fluid (amount not reported), delivery	7	Twin A, 530 gm (stillborn) Twin B, 430 gm (stillborn)	5
5	Percutaneous drainage of the fetal cyst and continuous amniotic diversion shunt- ing of the cyst	49	2730 Gm. Right uretero- nephropexy for uretero- pelvic junction stenosis (Fifteenth day of life)	2 Days after percutaneous drainage of the fetal renal cyst
6	Amniocentesis (1100 ml), delivery	2	Twin A, 1440 gm Twin B, 850 gm (neonatal death at 20 days)	2

Comment

A review of the literature¹⁻⁴ disclosed that only five cases of obstructive renal failure associated with polyhydramnios had been described previously (Tables I and II). The overdistended uterus was the result of polyhydramnios associated with twin gestation in all but one case (Case 5). The gestational ages ranged from 21 to 34 weeks. All patients were oliguric and had elevated serum creatinine, blood urea nitrogen, and uric acid levels, when this information could be obtained. Conservative management was attempted in all cases with twin gestations, but transabdominal amniocentesis relieved neither the oliguria nor the azotemia and pro-

vided only minimum temporary symptomatic relief. The prolongation of pregnancy in four of the five patients with twin gestations ranged between 2 and 3 days, and only in Case 4 was the pregnancy prolonged for 7 days. An interesting observation is that amniotomy and delivery produced a rapid return to normal of urine output, blood urea nitrogen, and serum creatinine. When delivery occurred within 2 to 3 days after admission (Cases 1, 2, 3, and 6), the serum creatinine and blood urea nitrogen returned to normal within 2 to 4 days after evacuation of the uterus. In Case 4, the patient was managed conservatively for 7 days and improved only after delivery. The serum creatinine and

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blood urea nitrogen levels at the fifth postpartum day were reported as 91 mmol/L and 5 mmol/L, respectively (equivalent to 1 mg/dl and 30 mg/dl, respectively). These values are slightly elevated, but no further follow-up is reported. This might suggest that delay in delivery may be related to slower return of renal function. Fetal outcome is usually related to the duration of gestation at the onset of polyhydramnios and renal failure. The perinatal death rate was 45.4% (four stillborn infants and one neonatal death, and all related to extreme prematurity).

In antepartum management, one should keep in mind that the perinatal mortality rate in patients treated with repeated amniocentesis is the same as that in untreated patients. Since complete bilateral ureteral obstruction causing anuria has been successfully treated by indwelling ureteral catheters, this therapeutic modality may play an important role in future cases; however, at the first sign of deterioration of the renal function, delivery should be the treatment of choice.

In summary, acute renal failure, secondary to an overdistended uterus, should be added to the causes of renal failure in pregnancy, particularly if the pregnancy is complicated by polyhydramnios and twins. Such patients should be advised to report if oliguria develops, and serum creatinine and blood urea nitrogen levels should be determined. This monitoring should be undertaken even in the absence of respiratory embarrassment, since three of the six patients did not have such symptoms (Cases 3, 4, and 5).

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Use of the Deaver retractor for exteriorization of ovarian cysts at laparotomy

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A method for the delivery of large benign ovarian cysts through the abdominal incision with use of the Deaver retractor is described. This method has facilitated the intact delivery of 11 large benign ovarian cysts in cases in which the abdominal incision was relatively small without the need of extending or modifying it. (AM J OBSTET GYNECOL 1985;152:885-6.)

Key words: Deaver retractor, large ovarian cysts

Occasionally when laparotomy is performed in a patient with a large benign ovarian cyst, it is difficult to deliver it intact through the abdominal incision. This may occur when Pfannenstiel's incision as well as when the low midline vertical abdominal incision is used. Obviously when malignancy is suspected, the vertical incision should be chosen and extended as necessary. When malignancy is not suspected, the surgeon is often reluctant to extend the incision, since its size, location, and shape are often of aesthetic importance, especially

to young women. Insertion of the hand in any attempt to exteriorize the cyst only further limits the diameter of the available space offered by the gaping incision. Grasping of the cyst capsule with instruments or use of sutures for traction may cause rupture and spillage of its contents into the peritoneal cavity. In these instances the use of the Deaver retractor blade for intact delivery of the cyst has been found to be of great assistance.

After entry into the abdominal cavity, the retractor blade, with its concave surface facing downward, is inserted by guiding it over an arc under the cyst (Fig. I). When the blade is properly positioned, the cyst is delivered by gentle upward traction. The width of the retractor is chosen according to the size of the cyst and the incision. Since the Deaver retractor is thinner than the surgeon's hand, it occupies less space, thereby fa-

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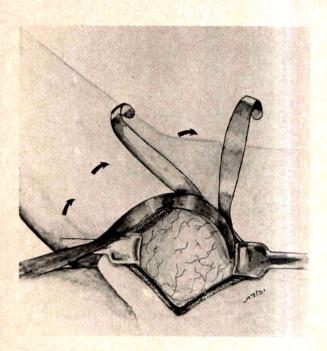


Fig. 1. Insertion of the Deaver blade under the cyst.

cilitating the delivery of the cyst. The blunt edges of the Deaver blade minimize the risk of injury to adjacent organs, and the even pressure exercised by it on a relatively large surface of the cyst reduces the chance of rupture. This method has been successfully used by us for exteriorization of 11 large benign ovarian cysts in cases in which the abdominal incision was relatively small without the need of extending or modifying it.

Eight of the patients had a mucinous cystadenoma, two a serous cystadenoma, and one a large benign cystic teratoma. The abdominal cavity was entered through a Pfannenstiel incision in nine patients and through a low vertical midline incision in the remaining two patients. In all patients the cyst was delivered intact without injury to adjacent tissues.

Recently the use of the soft silicone obstetric vacuum cup has been reported¹ to facilitate delivery of large pelvic masses at operation. Although this method seems to be effective for solid tumors, it may cause rupture when large cystic masses are encountered. Though we have used the Deaver blade only for exteriorization of ovarian cysts, it presumably may be used also for the delivery in an operation of solid pelvic masses.

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Small bowel obstruction secondary to a prior Moschcowitz procedure

Jeffrey M. Dicke, M.D.

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The Moschcowitz procedure has been advocated for the correction of large enteroceles. Documented complications of this technique are rare. Following is a review of this procedure with the presentation of a unique delayed complication. (AM J OBSTET GYNECOL 1985;152:887-8.)

Key words: Moschcowitz procedure, enterocele, hernia, bowel obstruction

In 1912 Dr. Alexis Moschcowitz first described his technique for cure of prolapse of the rectum via the placement of concentric purse-string sutures passed circularly about the cul-de-sac of Douglas, resulting in obliteration of this space. This procedure has subsequently been recommended for the abdominal surgical treatment of large enteroceles, as well as those occurring in women who have undergone prior pelvic or vaginal operations, in which case a vaginal approach to enterocele repair may be contraindicated.

Reported complications of this procedure are infrequent; two cases of rectal perforation² repaired at the time of operation and one case of a postoperative rectal hemorrhage² successfully managed conservatively have been described following synchronous abdominoperineal repairs, including Moschcowitz procedures, of large enteroceles. Theoretic complications, as elaborated by Dr. Moschcowitz, include operative insults to the ureters and hypogastric vessels as well as residual hernial defects secondary to incomplete peritoneal approximation.

A review of the literature has disclosed no previous reports describing the occurrence or clinical implications of such peritoneal defects. The following is a case of herniation and near strangulation of a segment of small intestine in a patient 1 year after a total abdominal hysterectomy, Moschcowitz procedure, and rectocele repair.

Case report

B. W., a 39-year-old black woman, entered the Medical Center Hospital emergency room with a 2-week history of increasing bilateral lower quadrant abdom-

inal pain. On the day of admission the patient had presented with severe generalized abdominal pain, which was exacerbated by any change from a lateral recumbent position. The patient denied having other systemic symptoms. The patient's past surgical history was significant for a total abdominal hysterectomy, Moschcowitz procedure, and rectocele repair (performed for uterine myomas, pelvic relaxation, and "a very deep cul-de-sac") I year prior to admission.

On examination the patient was an obese black woman in severe distress with blood pressure of 140/ 100 mm Hg, pulse rate 72 bpm, respiration rate 20 breaths per minute, and temperature 98.8° F. Abdominal examination revealed bilateral lower quadrant tenderness with mild to moderate guarding and no rebound; no masses were palpated and the abdomen was nondistended. Bimanual examination revealed an exquisitely tender, cystic cul-de-sac mass, 6 cm in diameter, which acutely exacerbated the patient's symptoms when palpated. The remainder of the physical examination was unremarkable. Pertinent laboratory data included the following: white blood cell count 5700 with 51 segmented granulocytes and 48 lymphocytes; hemoglobin, 13.7 gm/dl; hematocrit, 42.4%. Liver function tests, electrolytes, blood urea nitrogen, creatinine, and coagulation profile values were all within normal limits. An abdominal x-ray film revealed dilated loops of small bowel with air-fluid levels. Primary diagnostic considerations entertained at this time included adnexal torsion, cystic dilatation of the ovary with possible leakage, and/or entrapment by pelvic adhesions and small bowel obstruction.

In deference to the impressive symptoms elicited by manipulation of the cul-de-sac mass, the decision was made to proceed with an exploratory laparotomy. Subsequent exploration revealed a portion of small bowel incarcerated through a peritoneal defect at the apex of the previous Moschcowitz procedure. Lysis of the peritoneum at this site released a 10 cm segment of cyanotic small bowel which had herniated through this defect and was entrapped behind the peritoneum, which had been incorporated into the prior Moschcowitz repair. Intraoperative consultation was obtained with the gen-

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Reprint requests: Jeffrey M. Dicke, M.D., Department of Obstetrics and Gynecology. The University of Texas Health Science Center at San Antonio, 7703 Floyd Curl Drive, San Antonio, TX 78284. eral surgery staff, who felt the entrapped segment was viable and recommended it be left in situ. The peritoneum at the site of the prior Moschcowitz repair was reapproximated with a running suture, taking care to eliminate the defect. The remainder of the intraoperative findings were unremarkable, and the patient's postoperative course was uneventful.

Comment

This report illustrates that in the performance of a Moschcowitz procedure, care must be taken not only to avoid ureteral injury, but to ensure total obliteration of the cul-de-sac hernia, a matter of less immediate consequence but with potentially remote sequelae. Prevention of such a hernial defect is facilitated by ensuring that closure of the cul-de-sac is complete and that the peritoneum is approximated without undue tension

on the purse-string sutures and by taking care to include the serosa covering the rectum in the purse-string sutures. As demonstrated by this case, when confronted by a patient who is presenting with signs and symptoms compatible with a small bowel obstruction and who is known to have undergone a previous Moschcowitz procedure, one should be alert to the possibility that a residual peritoneal hernial defect may be responsible for such an event, especially in a patient with no other risk factors for bowel obstruction.

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Microvasculature of preovulatory follicles: Comparison of in situ and in vitro perfused rabbit ovaries following stimulation of ovulation

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Perifollicular vasculature undergoes significant morphologic changes as ovulation approaches. These vascular changes were observed in in vitro perfused and in situ rabbit ovaries by means of scanning electron microscopy of microcorrosion casts. Casts were made in in situ unstimulated ovaries, in situ ovaries stimulated with human chorionic gonadotropin, in vitro perfused unstimulated ovaries, and in vitro perfused ovaries after an ovulation-inducing dose of human chorionic gonadotropin, prostaglandin F_{2n}, histamine, or norepinephrine. Dilated vessels, extravasation of resin from weakened vessels, and filling defects at the apex of the follicle were observed in in situ ovaries 9 to 12 hours after stimulation and in in vitro perfused ovaries 4 to 6 hours after human chorionic gonadotropin. In vitro perfused ovaries stimulated with prostaglandin F_{2n} or histamine demonstrated dilated capillaries with extravasation of the resin and filling defects at the apex of large follicles. Norepinephrine-stimulated ovaries showed incomplete filling of vessels, although some large follicles showed extravasation of resin. The occurrence of dilated vessels, extravasation of resin, and filling defects is indicative of preovulatory vascular changes in both in situ and in vitro perfused ovaries, regardless of the ovulatory stimulus. (AM J OBSTET GYNECOL 1985;152:889-95.)

Key words: Ovarian morphology, ovulatory changes, microvasculature, scanning electron microscopy

The rabbit follicle is surrounded by a complex capillary network located between the theca interna and the avascular membrane granulosa.¹⁻⁴ As the time of ovulation is approached, the perifollicular vasculature undergoes significant morphologic changes, including an increase in vascular permeability.^{1-4,5} The in vitro perfused rabbit ovary serves as an effective model by means of which the effects of various agents on the

follicular vascular system can be investigated. With the use of this perfusion model, ovulation can be consistently induced by human chorionic gonadotropin (hCG) or other stimulatory agents in an ovary isolated from systemic influences and perfused with a chemically defined medium.⁶⁻⁹

Scanning electron microscopic observation of vascular casts was initially used by Murakami¹⁰ to study the capillary network of the rat renal glomeruli. Now, with improvements in the technique for preparation of vascular casts, this procedure can be applied to the investigation of various mammalian microcirculatory systems. ¹¹⁻¹⁴ Previously, Kanzaki et al. ⁴ reported on the characteristic vascular patterns of rabbit follicles at various stages of development after the administration of human chorionic gonadotropin.

The present study was undertaken for two purposes: (1) to elucidate alterations in perifollicular vasculature in in situ and in vitro perfused ovaries prior to ovulation and (2) to compare these features with those observed under experimental conditions which are known to induce ovulation in this perfusion system.

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Fig. 1. In situ ovary from an unstimulated rabbit demonstrating the multilayered vascular network of a follicle (×150). Scale indicates 100 μm.

Material and methods

Twenty sexually mature New Zealand white female rabbits were used in these experiments. The rabbits were isolated for a minimum of 3 weeks prior to the experimental procedure under controlled temperature and light with free access to Purina Rabbit Chow and water.

In situ ovaries. Microvascular casts were made of in situ ovaries of unstimulated rabbits and rabbits treated with 100 IU of hCG via marginal ear vein. At laparotomy, ovaries were isolated, anastomotic connections to the ovary were ligated, and the ovarian artery was cannulated. The laparotomy was performed on hCGtreated rabbits at 3, 6, 9, or 12 hours after administration of hCG. Four ovaries from two rabbits were studied at each time interval. Four unstimulated ovaries were obtained from two rabbits not treated with hCG. In the rabbit, ovulation characteristically occurs 10 to 12 hours after intravenous administration of hCG. Ten milliliters of Mercox unsaturated resin (Ladd Research Industries, Burlington, Vermont) was mixed with 0.25 gm of resin hardener immediately prior to use. After the injection of 20 ml of perfusion medium to wash out circulating blood, the Mercox resin was slowly injected with minimal pressure through the ovarian arterial cannula until the resin flowed from the ovarian vein.

In vitro ovaries. In vitro perfused rabbit ovaries were prepared as previously described. ^{6, 15} Briefly, after cannulation of the ovarian artery as described above, the ovary, artery, and vein with supporting adipose tissue were removed and placed in an in vitro perfusion system. The ovary was perfused with 150 ml of oxygenated

tissue culture medium (M199, M. A. Bioproducts, Bethesda, Maryland) supplemented with insulin, heparin, streptomycin, and penicillin. The perfusion system, consisting of oxygenator, pulsatile pump, and perfusion chamber, functioned in a constant-temperature room, at 37° C. In each experiment, one of the following agents was added to the perfusion medium at the onset: hCG (50 IU), prostaglandin $F_{2\alpha}$ (100 ng/ml), histamine (100 ng/ml), or norepinephrine (6 \times 10⁻⁷ mol/ L). Histamine was also added hourly and norepinephrine at 30-minute intervals in the same dosages as used initially. These agents in the dosage schedules cited have been shown to induce ovulation in the in vitro perfusion system.⁶⁻⁹ Saline solution (0.1 ml) was also added at the onset of perfusion in the unstimulated control group. At 4 hours after initial treatment, the Mercox resin was injected through the arterial cannula of one ovary of each pair with the use of minimal pressure until the resin flowed from the ovarian vein. At 6 hours, the arterial system of the contralateral ovary, which was treated with the same agent, was injected with Mercox. Two rabbits (four ovaries) were used in this fashion for each treatment.

After injection with Mercox, the ovaries from both the in situ and in vitro preparations were removed and kept at 40° to 50° C for 1 hour to allow the Mercox to harden. Each ovary was then placed in 20% potassium hydroxide for 3 to 4 days to degrade the ovarian tissue. The resultant cast was carefully washed under tap water and then air dried. The cast was mounted onto a specimen holder with carbon paint, coated with 100 to 150 Å gold palladium in a Polaron scanning electron mi-

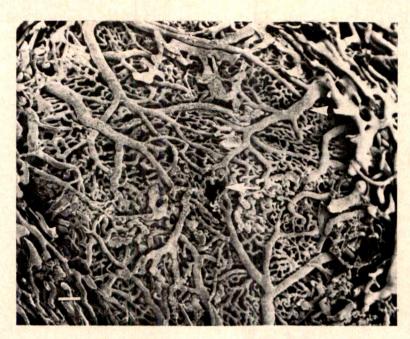


Fig. 2. In situ ovary 6 hours after hCG stimulation demonstrating a monolayer vascular network in a follicle (1.4 mm in diameter) and vessel dilatation (arrowhead). Note the small filling defect at the apex (arrow) (×70). Scale indicates 100 μm.

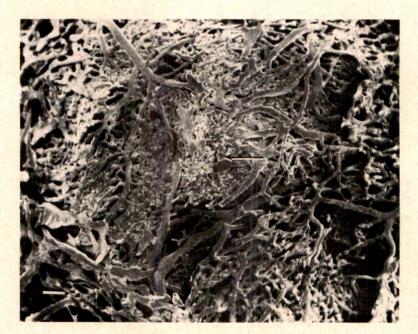


Fig. 3. In situ ovary 9 hours after hCG stimulation demonstrating dilation of the follicular capillaries and extravasation of resin at the follicle apex (arrow) (×70). Scale indicates 100 μm.

croscope sputter-coating unit. Observations were made with an Amray 1200 B scanning electron microscope operated at 5 to 15 kV.

The time at which the ovarian arteries were injected with Mercox was chosen to enable progressive examination of the vasculature of preovulatory follicles. Our previous experience with use of the in vitro perfused ovary system showed that the time of ovulation after hCG or another stimulatory agent is characteristically accelerated. Thus perfused ovaries were injected with Mercox at 4 and 6 hours, and in situ ovaries were injected at 3, 6, 9, and 12 hours after administration of hCG.

Results

In situ ovaries. The unstimulated in situ rabbit ovary demonstrated many basketlike networks of capillaries surrounding follicles 200 to 600 µm in diameter (Fig.



Fig. 4. In vitro perfused ovary 6 hours after addition of hCG to the perfusate demonstrating irregular filling defects (arrow) and resin extravasation (arrowheads) (×80). Scale indicates 100 μm.



Fig. 5. In vitro perfused ovary 4 hours after addition of prostaglandin $F_{2\alpha}$ to the perfusate demonstrating flattening and dilatation of vessels with a filling defect (*arrow*) at the apex (×70). Scale indicates 100 μm.

1). Arterioles, capillaries, and venules of each folliele were completely filled with the polyester resin and were arranged as microcirculatory units separate from the stromal circulation. The follicular vasculature formed a complex multilayered network.

Maturing follicles increased in size after the administration of hCG; however, no changes were evident in the vascular casts at 3 hours after hCG. At 6 hours after hCG, the follicular vessels formed monolayer vascular

networks with an associated increase in follicle size (1.2 to 1.6 mm in diameter) (Fig. 2). Dilatation of follicular vessels was also observed.

Nine hours after hCG, the follicular capillaries were dilated, and irregular defects in the region of the follicle apex could be observed. Extravasation, as demonstrated by widespread resin leakage into the intercapillary spaces, was observed at the apex of preovulatory follicles (Fig. 3). By 12 hours after hCG, the intrafol-

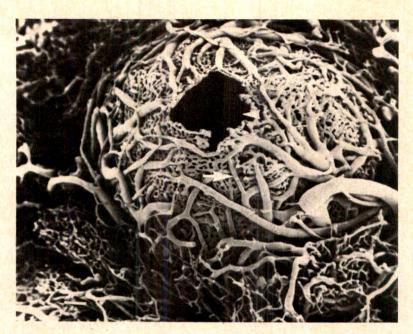


Fig. 6. In vitro perfused ovary 4 hours after the initial exposure to histamine demonstrating dilated vessels with a filling defect at the apex (arrowhead). Note the arterial venous shunt (arrow) (×70). Scale indicates 100 µm.

licular space of some follicles contained extravasated resin, the defects in the apical region had increased in size, and the basketlike architecture was no longer evident.

In vitro ovaries. Follicular vessels of the unstimulated in vitro perfused ovary were not dilated and appeared as multilayered vascular networks. After 4 hours of perfusion, no remarkable difference in the appearance of the follicular microvasculature was observed. The follicular vascular network remained intact under the conditions of perfusion in the absence of any stimulation. The vascular network during 4 hours of ovarian perfusion was similar to that observed in situ without hCG, as seen in Fig. 1.

After ovarian perfusion with hCG for 4 hours, the follicular vessels appeared markedly dilated as compared to vessels in nonfollicular regions of the same ovary. Six hours after hCG, irregular filling defects and resin leakage were observed at the apical region of follicles (Fig. 4). The microvascular changes in the perfused ovaries after hCG were observed at an accelerated time scale (6 hours) compared to the in situ ovaries (9 hours).

Four hours after prostaglandin F_{2α} stimulation, ovaries showed marked vessel dilatation and filling defects at the apex of the preovulatory follicles similar to that observed after hCG administration in vitro (Fig. 5). At 6 hours after administration of prostaglandin F_{2α}, extravasation of resin could be seen at the apical region of the follicle. Extravasation of resin was extensive and invaded the follicular cavity.

In histamine-stimulated ovaries, as the follicles developed, follicular vessels, which formed monolayer networks, were markedly dilated (Fig. 6). At 6 hours after histamine, extravasation of resin was observed.

Follicular capillaries of norepinephrine-stimulated ovaries were less dilated than those of ovaries perfused with hCG, prostaglandin F_{2a}, or histamine. The irregular filling defects of the microvascular casts were observed not only at the apex, but also in the lateral regions of the preovulatory follicle (Fig. 7). Widespread leakage of resin was observed over the entire follicle. Follicular vessels were flattened in comparison to the vessels of ovaries treated with other stimulants.

Comment

This study demonstrated the alterations in the microvasculature of preovulatory rabbit follicles in the in situ ovary and the in vitro perfused ovary stimulated with various agents. Microcorrosion casts prepared with a polyester resin used for these scanning electron microscopic observations met several criteria: (1) The vascular system was completely filled by the resin; (2) the cast polymerized and dried without shrinkage; (3) the supporting tissue was thoroughly macerated.11, 12

The in vitro perfused rabbit ovary has made possible the serial study of the ovulatory process under isolated and regulated conditions. Previous studies have confirmed that ovulated ova recovered in this system and fertilized in vivo or in vitro develop into normal offspring.16,17 The perifollicular vascular changes are identical in vitro and in situ, although the rate at which they

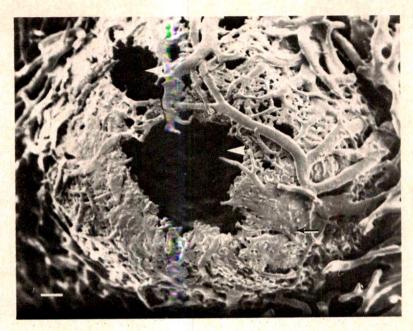


Fig. 7. In vitro perfused ovary 4 hours after initial exposure to norepinephrine demonstrating both apical and lateral filling defects (*arrowheads*) and extravasation of resin (*arrow*) (×70). Scale indicates 100 µm.

occur is accelerated in vitro. This observation further supports the usefulness of the in vitro model for studying perifollicular vascular morphologic changes under various experimental conditions.

The characteristic alterations in the rabbit perifollicular vasculature observed by means of microcorrosion casts prior to ovulation include vasodilatation, widespread leakage of resin, and filling defects at the periapical region of the preovulatory follicle. Marked dilation of the periapical capillaries and venules and extravasation of resin could be noted by 4 hours after the administration of either hCG, prostaglandin F₂₅, or histamine.

The involvement of prostaglandins in the regulation of ovulation has been described in several species, including the rabbit, 9 rat, 18,19 and rhesus monkey. 20 In the rabbit, the intrafollicular levels of prostaglandins F and E increase markedly just prior to the time of ovulation. Furthermore, the observations that exogenous prostaglandin $F_{2\alpha}$ could induce ovulation in the perfused rabbit ovary and that inhibition of prostaglandin synthesis prevents ovulation suggest that prostaglandin $F_{2\alpha}$ plays a significant role in ovulation. It has been confirmed that the vasodilatation activity of prostaglandins is responsible for their ability to potentiate exudation. 21,22 These changes in the vascular system caused by prostaglandins may be related to perifollicular edema associated with ovulation. 21

Ovarian histamine may also play a physiologically significant role during ovulation.⁸ In the study cited, the addition of histamine to the perfusate induced ovu-

lation, whereas the antihistamine cimetidine inhibited histamine-induced ovulation in the perfused rabbit ovary. Several investigations suggest that the principal effect of histamine may be to increase vascular permeability.21,22 Vasodilatation and extravasation of resin observed in the perfused ovaries treated with prostaglandin F20 or histamine suggest that prostaglandin F20 and/or histamine may achieve ovulation by increasing vascular permeability in the wall of the preovulatory follicle. These results are consistent with those of other reports in that, along with vasodilatation, there is an increase in vascular permeability in hCGstimulated follicles approaching the time of ovulation in situ. 12. 23 It is unlikely that extravasation of resin is due to injection under pressure, since it is observed in the in situ follicular vessels only 9 to 12 hours after administration of hCG. Extravasation of resin may be secondary to an increase in vascular permeability and/ or to a partly disrupted capillary wall.12

Immediately prior to anticipated ovulation, resin failed to enter the apical vessels. This finding was observed in both stimulated in situ ovaries and in vitro perfused ovaries. Of interest is the fact that the appearance of the filling defect was quite different in the norepinephrine-stimulated ovaries. In general, the presence of filling defects may be attributable to microthrombus formation or to the presence of arteriovenous shunts. Filling defects observed in the periapical region, as seen with norepinephrine, indicate a functional avascular area which may exert an important effect on follicular hemodynamics prior to ovulation.

The observation of filling defects both at the apex and lateral regions of the follicle wall in norepinephrine-treated ovaries suggests that norepinephrine may create an ischemic change in the preovulatory follicle, weakening it and facilitating rupture. Arteriovenous shunts may also cause hemodynamic changes which may be involved in the process of follicle rupture.

In conclusion, these observations indicate that rabbit preovulatory vascular changes are comparable in the in situ ovary stimulated by hCG and in the in vitro perfused ovary, regardless of the ovulatory stimulus. Prior to follicle rupture, dilated vessels and extravasation of resin may be associated with increased vascular permeability. The presence of filling defects suggests a functional avascular area at the periapical region which may weaken the follicle wall and facilitate rupture.

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Origin of immature teratoma of the ovary

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Six cases of immature teratoma of the ovary were karyotyped and analyzed for chromosomal heteromorphisms, enzyme polymorphisms, and HLA specificities to determine their mechanism of origin. Three cases were chromosomally abnormal, with karyotypes of 48,XX, +14, +21; 47,XX, +20; and 47,XXX, respectively. The tumors with 48,XX, +14, +21 and 47,XX, +20 karyotypes were heterozygous for chromosomal heteromorphisms which were identical to those of their host and therefore originated from a premeiotic cell or failure of meiosis I. Both had a poor progmosis. The 47,XXX tumor and the three cases with normal karyotypes were homozygous for chromosome heteromorphisms and either homozygous or heterozygous for enzyme and HLA markers and therefore originated from a failure of meiosis II or duplication of a mature haploid ovum. All four had an uneventful postoperative course. These observations show that immature teratomas are like cystic teratomas in having at least three separate mechanisms of origin. However, they are unlike cystic teratomas in having a high proportion with chromosome abnormalities and a high rate of malignant transformation. (Am J Obstet Gynecol. 1985;152:896-900.)

Key words: Immature teratoma, chromosome, genetic marker, malignant transformation

Teratomas of the ovary are divided into two categories, cystic teratomas (mature teratomas) and ammature teratomas. Cystic teratomas, which are composed entirely of mature tissues originating from three germ cell layers, the ectoderm, mesoderm, and endoderm, are the most common ovarian tumors in young women and have a benign behavior except for malignant degeneration of mature elements in the tumor. Immature teratomas, representing approximately 1% of ovarian teratomas, contain immature elements from three germ cell layers and have a high propensity for malignancy, with only 30% to 60% of patients surviving for 2 years after diagnosis without evidence of malignant transformation.

Recent genetic studies have shown that cystic teratomas have a 46,XX normal female karyotype and that chromosome and enzyme markers of the tumors are different from those of their hosts. Linder et al.² found that chromosomal heteromorphisms of cystic teratomas were homozygous and enzyme polymorphisms were either homozygous or heterozygous, and from these findings they postulated that cystic teratomas were derived from a germ cell after meiosis I by suppression of meiosis II or by fusion of polar body II with the

oocyte. Carritt et al.³ found four ovarian teratomas showing a heterozygous pattern of chromosomal heteromorphisms and proposed a failure of meiosis I as a second mechanism for the origin of ovarian teratomas. Parrington et al.⁴ studied 21 teratomas and found that 11 of 13 teratomas that were homozygous for chromosomal heteromorphisms were also homozygous for all enzyme polymorphisms. In order to explain the excess of complete homozygosity they postulated a duplication of a mature ovum as a third mechanism for the origin of teratomas.

If it is assumed that malignancy of the teratoma is associated with homozygosity of a mutant gene (or genes), immature teratomas that become malignant might be expected to originate largely or wholly by duplication of an ovum rather than by failures of meiosis I and II. However, because of their relative rarity, mechanisms of origin of immature teratomas have not been studied. The present study was undertaken in order to elucidate the chromosomal constitution and the mechanism of origin of immature teratomas of the ovary by analyses of chromosome heteromorphisms, enzyme polymorphisms of phosphoglucomutase I and esterase D, and major histocompatibility leukocyte antigen (HLA) specificities. In this paper we will deal with the malignant potential of immature teratomas in terms of chromosomal constitution and gene homozygosity.

Material and methods

Six cases of immature teratomas of the ovary shown in Table I were examined, of which detailed clinical

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Table I. Clinical and pathologic features of six cases

Case No.	Age (yr)	Clinical stage	Histologic grade*	Prognosis (after operation)	
1	10	ľΔ	5)	NED at 8 yr, 8 mo	
9	19	йC	3	Died, 2 mo	
$\bar{3}$	30	IA	i i	NED at 5 yr, 8 mo	11 1444 1014
4	28	IA	3	Recurrence 8 mo later	
		Recurrent tumor	2	NED at 4 yr, 8 mo	
5	38	IA	3	NED at 2 yr, 11 mo	
6	35	IA	$1 \sim 1^{-1}$, 2^{-1}	NED at 1 yr, 8 mo	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1

NED = No evidence of disease.

Table II. Mode of inheritance of heteromorphic features in immature teratomas of the ovary

				Chr	omosom	al hete	romorph	iisms*		F	ILA	Enzyme va	riants
Case No.	Tissue	Karyotype	3	4	13	14	15	21	22	A	В	Phosphogluco- mutase 1	Esterase D
1 2 3 4 5	Host Tumor† Host Tumor† Host Tumor† Host Tumor‡ Host Tumor‡ Host Tumor† Host	46.XX 46.XX 46.XX 48.XX, +14, +21 46.XX 46.XX 46.XX 47.XX, +20 46.XX 47.XXX 46.XX	aa aa aa aa aa aa aa aa aa ab bb	aa aa aa aa aa aa aa aa aa	ab aa aa aa aa aa ab ab aa aa aa aa aa a	aa aa aa aa aa aa aa aa aa	aa	ab bb aab aa ab aa aa aa	ab bb aa aa aa aa aa ab ab ab	9,11 9,9 9,w31 9,w31 49,w31 w24,w24 w24,w24	w60,w54 w54,w54 w51,w54 w51,w54	2-1 2-2 2-1 2-1 2-1 2-1 2-1	2-1 1-1 1-1 1-1 1-1 1-1

Solid-line boxes, A marker transmitted in duplicate to the teratoma. Dashed line boxes, Both markers transmitted to the teratoma.

findings were reported elsewhere.⁵ Preoperative diagnosis of ovarian tumor was made by pelvic and ultrasonographic examination in four cases with a chief complaint of abdominal mass, and in two cases tumors were found incidentally during pregnancy and the puerperium by regular pelvic examination. At operation, tumors were localized within an affected ovary in five cases (International Federation of Gynecology and Obstetrics Stage IA), but metastases to the uterus and pelvic peritoneum with ascites were observed in one case (Stage IIC, Case 2). In one of the five cases in Stage IA (Case 4) a second operation was required 8 months after the first operation because of intraperitoneal tumor recurrence. Follow-up study of the patients showed that five patients were alive but that the patient with Stage HC disease had died 2 months after operation.

Tissues taken from three different sites in the solid portions of the tumors were washed in sterile Ringer's solution, minced with scissors, and cultured in flasks (Falcon 3013) containing 5 ml of Eagle's minimum essential medium supplemented with 20% fetal bovine serum. When sufficient growth of fibroblast-like cells

was observed, the culture surface of the flask was gently scratched with a pipette to make a free cell suspension. A portion of the cell suspension was transferred to a Leighton tube containing a glass slide. After 2 or 3 days of additional culture, cells were treated with colcemid, hypotonic solution (equal volumes each of 0.075 mol/L of potassium chloride and 1% sodium citrate), and acetic methanol and air-dried. Chromosome preparations of peripheral blood lymphocytes from the patients were made by standard procedures and harvested after 72 hours' culture. Some of the slides were treated with trypsin and stained with Giemsa for G-banded analysis, and others were stained in quinacrine mustard for Q-banded heteromorphisms and in chromomycin A₅ and methyl green for R-banded heteromorphisms.⁷

Cell suspensions from the tumors and peripheral blood lymphocytes from the patients were used for typing of HLA-A and HLA-B specificities by the standard National Institutes of Health microcytotoxicity method.⁸ Enzyme polymorphisms of phosphoglucomutase 1 and esterase D were analyzed by horizontal starch gel electrophoresis with the use of extracts of

^{*}Histologic grade by Norris et al.6

^{*}a and b are symbols and do not represent specific heteromorphism.

[†]Primary tumor.

[‡]Recurrent tumor.

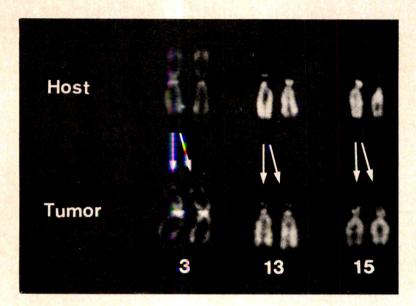


Fig. 1. Q-banded chromosomes 3, 13, and 15 from Case 6. Q heteromorphisms of homologous chromosomes of the tumor showed homozygous pattern transmitted from one of the homologous chromosomes of the host.

culture cells of the tumors and red blood cell hemolysates of the peripheral blood. 9, 10 These genetic markers had previously been shown by us to be reliable indicators of the origin of hydatidiform moles 11 and cystic teratomas of the ovary. 12

Results

The results of analyses of karyotypes and chromesome, HLA, and enzyme markers of six teratomas and their hosts are summarized in Table II. All six patients (hosts) had normal 46,XX chromosomal constitutions.

Three teratomas showed a 46,XX normal female karyotype, which was determined to be of tumor cell origin from the findings of chromosome heteromorphic patterns of the culture cells that were different from those of their hosts. The remaining three teratomas showed nonmosaic numerical abnormalities in analysis of more than 50 cells from multiple cultures, that is, 48,XX, +14, +21 (Case 2), 47,XX, +20 (Case 4), and 47,XXX (Case 5).

Chromosomal heteromorphisms of the teratomas appeared completely homozygous in four cases (Cases 1, 3, 5, 6) (Fig. 1), whereas at least one pair of chromosomes in the hosts were clearly heterozygous. In three, HLA specificities and enzyme polymorphisms were analyzed and found to be homozygous in one teratoma (Case 3) and heterozygous in two teratomas (Cases 5 and 6). Chromosomal heteromorphisms are markers at, or very close to, the centromere, so that crossing over between the centromere and the heteromorphic bands is negligible. Therefore teratomas with complete homozygosity for Q- and R-heteromorphisms are considered to originate from a germ cell subsequent to

completion of meiosis I either by suppression of meiosis II (or fusion of second polar body with an ovum) or by duplication of a mature ovum. In tumors originating by the former mechanism, HLA and enzyme markers are either homozygous or heterozygous depending on whether crossing over between the loci in question and the centromere has occurred. However, in tumors originating by duplication of a mature ovum, all markers are homozygous. Thus Cases 5 and 6 were considered to result from failure of meiosis II while Cases 1 and 3 could have resulted from failure of meiosis II or duplication of a mature ovum.

The remaining two teratomas (Cases 2 and 4) were heterozygous for chromosomal heteromorphisms (Fig. 2), and in case 4 HLA and enzyme markers also showed a heterozygous pattern identical to that of the host. The possibility of contamination with maternal cells in both could be excluded because cultures established from the teratomas in three separate flasks showed the same chromosomal abnormality in all the cells observed. Teratomas with heterozygous chromosomal heteromorphisms can arise by one of two mechanisms. The first is suppression of meiosis I followed by separation of chromatids of each homologous chromosome into two daughter cells at meiosis II. This will result in some enzymes being homozygous that are heterozygous in the host as a result of crossing over at meiosis I and segregation at meiosis II. The second mechanism is mitotic division of a premeiotic cell, by which all the genetic markers that are heterozygous in the host will also be heterozygous in the tumor. Although homozygosity of HLA or phosphoglucomutase 1 markers was not seen in the tumor in Case 4, the only case tested,

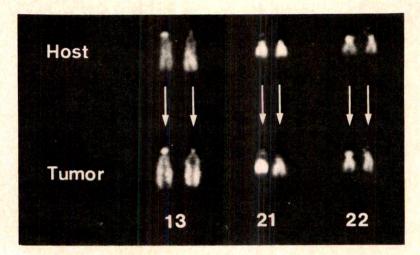


Fig. 2. Q-banded chromosomes 13, 21, and 22 from Case 4. Q heteromorphisms of homologous chromosomes of the tumor showed heterozygous pattern similar to those of the host.

and the exact mechanism of origin in this case and in Case 2 cannot be determined because of the small number of loci tested. If Cases 2 and 4 arose by suppression of meiosis I, their aneuploid constitution must have arisen as a result of nondisjunction at meiosis II or the first postmeiotic division with subsequent loss of the resulting hypodiploid cell line. However, if Cases 2 and 4 arose from a premeiotic cell, their aneuploid constitution must have arisen during mitotic division of the oogonium. The aberrant behavior of the germ cell may be a direct consequence of its abnormal chromosomal constitution.

Comment

Cytogenetic studies of cystic teratomas of the ovary have shown that the vast majority have a normal 46,XX karyotype but a very few are mosaics.4.13 In our continuing study, 124 of 128 cystic teratomas have a 46,XX constitution and only four have numerical abnormalities, three trisomies and one triploidy. In contrast to cystic teratomas, very few cases of immature teratoma have been studied cytogenetically. Arias-Bernal and Jones¹⁴ reported an immature teratoma with trisomy 3 and Kusyk et al. 15 reported a trisomy 12. In the present series, three of six immature teratomas showed numerical abnormalities, double trisomies of 14 and 21, trisomy 20, and triple X. Although the total number of cases studied is still small, these reports indicate that immature teratomas are often associated with a chromosomal abnormality.

Hunt and Jacobs¹⁶ found trisomy 20 in a high proportion of in vitro cultures established from complete hydatidiform moles and speculated on the correlation of trisomy 20 with the potential for malignant transformation following hydatidiform mole. It is interesting that trisomy 20 was also found in an immature teratoma resulting from parthenogenesis; however, there was no consistency in additional chromosomes in trisomic and double trisomic immature teratomas reported thus far.

Ovarian teratomas arise from a germ cell in a number of different ways. In the present series, two of six immature teratomas arose from a germ cell at or before meiosis I. This frequency of meiosis I errors is similar to that seen in cystic teratomas, eight of 21 (38%) in the study by Parrington et al.4 and 25 of 61 (41%) in the study by Nomura et al. (unpublished data). These observations suggest that the origin of mechanisms of immature teratomas are similar to those of cystic teratomas.

The finding that the two immature teratomas that resulted from a premeiosis II error had a poor prognosis, while the four immature teratomas that arose by a meiosis II or post-meiosis II error were associated with an uneventful postoperative course, is suggestive in terms of the relationship between homozygosity of genes and malignant transformation of immature teratomas. Teratomas are similar to hydatidiform moles with respect to their origin and homozygosity of the genome. More than 90% of moles originate from the fertilization of an empty ovum by a haploid sperm, followed by its duplication without cytokinesis, leading to a diploid with complete homozygosity of the genes.¹⁷ The minority result from the fertilization of an anucleate egg by two haploid spermatozoa, resulting in heterozygosity for approximately half the genes that are heterozygous in the father.11 At the time when homogyzous moles were first ascertained, the high potential of moles for choriocarcinoma was considered to be associated with homozygosity of a recessive mutation of a gene (or genes) which controlled cell growth.18 However, data incompatible with this view have recently been reported by Wake et al. 19 and Davis et al., 20 who compared the incidence of postmolar sequelae between patients with homozygous moles and those with heterozygous moles and found malignant transformation to be more common among the heterozygous moles. Therefore, results obtained from both immature teratomas and hydatidiform moles suggest that, contrary to expectation, complete homozygosity is at a disadvantage by comparison with heterozygosity in terms of malignant transformation.

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Peritoneal fluid 6-keto prostaglandin $F_{1\alpha}$ levels in women with endometriosis

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Peritoneal fluid was collected from women undergoing investigations for infertility at laparoscopy performed during the luteal phase. The volume of fluid was recorded and concentrations of 6-keto prostaglandin $F_{1\alpha}$ were determined by radioimmunoassay. No difference was found in either the total amount or the concentration of 6-keto prostaglandin $F_{1\alpha}$ in the women with or without endometriosis. Furthermore, there was no difference in the volume of peritoneal fluid between these two groups of women. We conclude that 6-keto prostaglandin $F_{1\alpha}$ in peritoneal fluid is not associated with macroscopically visible endometriosis. (AM J OSSTET GYNECOL 1985;152:901-4.)

Key words: Endometriosis, 6-keto prostaglandin F_{la}, peritoneal fluid, infertility

There is considerable evidence to support an association between endometriosis and infertility. When the endometriosis is extensive and there is anatomic disorganization of the pelvis, such an association would seem to be readily explained on the basis of impaired ovum pickup. With lesser degrees of endometriosis such an association, if indeed one exists, is much more difficult to explain.

It has been suggested that prostaglandins produced by the ectopic endometrium may interfere with ovarian function.2.3 Prostaglandins are synthesized by endometrium4 and it has been demonstrated that high concentrations of prostaglandin F2a inhibit follicular growth in mice5 and cause luteolysis in humans6 and monkeys.7 Furthermore, prostaglandins and thromboxane A2 act on smooth muscle. If prostanoids are present in increased amounts in the peritoneum of women with endometriosis they may affect tubal function. Studies addressing this possibility have shown inconsistent results. It has been reported that the levels of prostaglandin E₂, prostaglandin F_{2α}, 15-keto-13,14- dihydro prostaglandin F2a, and thromboxane B2 are not elevated in the peritoneal fluid of women with endometriosis.8 Conversely, it has been reported that levels of thromboxane B₂ (the stable metabolite of thromboxane A₂) and 6-keto prostaglandin $F_{1\alpha}$ (the stable metabolite of prostacyclin) are elevated in the peritoneal fluid of women with endometriosis.3

In an attempt to resolve this issue, we have measured the concentration and determined the total amounts of 100
9050(|E| 4020100 NO ENDOMETRIOSIS

Fig. 1. Peritoneal fluid concentration of 6-keto prostaglandin F_{1a} . Horizonal lines indicate the median values.

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Reprint requests: Dr. M. J. James, Department of Surgery, Flinders Medical Centre, Bedford Park, South Australia 5042. 6-keto prostaglandin F_{1a} in the peritoneal fluid of 113 women who presented with infertility and who underwent laparoscopy as part of their investigation for this problem.

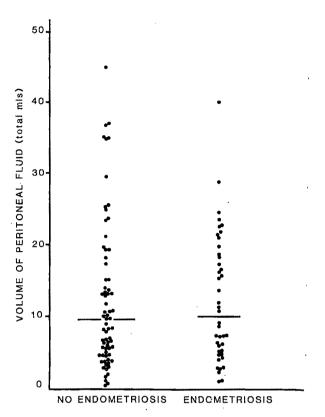


Fig. 2. Peritoneal fluid volumes. *Horizonal lines* indicate the median values.

Methods

Subjects. Women undergoing laparoscopy during the luteal phase of the menstrual cycle were studied. The majority of women belonged to infertile couples. These were divided into two clinical groups designated "endometriosis" and "nonendometriosis." A smaller group of patients undergoing laparoscopic sterilization during the luteal phase of the cycle and designated "fertile" was also studied. At laparoscopy, the total amount of peritoneal fluid lying free in the pouch of Douglas or in the uterine vesical pouch was aspirated into a syringe with a Verres needle through a second puncture. The diagnosis of endometriosis was made on the typical appearance of this condition, occasionally supplemented by biopsy and histologic information. The nonendometriosis group included women with adhesions from old pelvic inflammatory disease or operation or fallopian tube occlusion and those in whom the pelvis was normal. No woman in this study was having hormone treatment. The peritoneal fluid was stored frozen until subsequent assay of prostaglandin levels.

Prostaglandin assays. Aliquots (0.5 ml) of peritoneal fluid were acidified (1N hydrochloric acid, 100 μ l) and tritium-labeled 6-keto prostaglandin F_{1a} (0.005 μ Ci) was added. Prostaglandins were extracted with ethyl acetate. This solvent was evaporated with nitrogen and 0.5

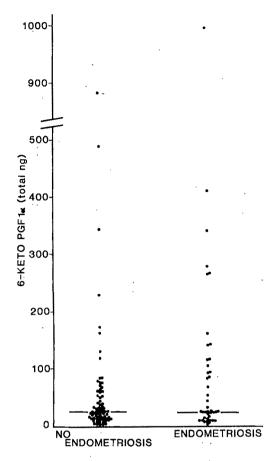


Fig. 3. Total amounts of peritoneal fluid 6-keto prostaglandin $F_{\rm la}$. Horizontal lines indicate the median values.

ml of sodium carbonate (100 μ mol/L, pH 10) was added to the residue. Aliquots (0.1 ml) of this solution were used to calculate the extraction efficiency and to estimate the amount of 6-keto prostaglandin $F_{1\alpha}$ by radio-immunoassay. The radioimmunoassay system used commercially available antisera (Seragen Inc., Boston, Massachusetts), tritium-labeled 6-keto prostaglandin $F_{1\alpha}$ (Amersham International, Sydney, Australia), and 6-keto prostaglandin $F_{1\alpha}$ (Cayman Chemical, Denver, Colorado). Determinations were usually performed in triplicate unless <1.5 ml of peritoneal fluid was collected.

Results

Endometriosis group versus nonendometriosis group. Peritoneal fluid concentrations of 6-keto prostaglandin $F_{1\alpha}$ and the volumes of collected peritoneal fluid were measured in 113 women attending the Fertility Clinic. Of this group, 42 women had evidence of endometriosis at laparoscopy and 71 women had no evidence of this disease. The concentrations of 6-keto prostaglandin $F_{1\alpha}$ in the two groups are represented in Fig. 1. The median values for the two groups were similar (Table I) and although the larger median value

Table I.	Median	values	of	peritoneal	fluid	volumes a	ınd	levels of	of (5-keto	prostagland	in F	7.

	6-Keto prosto		
Group	Concentration (ng/ml)	Total amount . (r.g)	Volume (ml).
Endometriosis $(n = 42)$	3.2	24.9	9.1
Nonendometriosis $(n = 71)$	2.4	25.1	9.0
Fertile $(n = 10)$	9.2	26.6	3.8

was only 3.2 ng/ml, the overall range was very large, extending from 0.8 to 98.2 ng/ml. The values for the two groups were compared by the Mann-Whitney U test (two-tailed) and no difference between the two groups was found ($\alpha = 0.05$).

Because prostaglandin concentration is a function of the volume of fluid and the total amount of prostaglandin present, we have also recorded these individual parameters. The distributions of peritoneal fluid volumes appeared similar in the two groups (Fig. 2) and the median values of the two groups were similar (Table I). When the peritoneal fluid volumes of the two clinical groups were compared (Mann-Whitney U test), no difference was found ($\alpha = 0.05$, two-tailed). The median values for the total amount of 6-keto prostaglandin F₁₀ present in peritoneal fluid were similar for the two groups (Table I), and although the median values were approximately 25 ng the combined range of values extended from 1 to 996 ng (Fig. 3). There was no difference in total prostaglandin levels between the two groups when compared by the Mann-Whitney U test $(\alpha = 0.05, \text{two-tailed}).$

With regard to the probability of a Type II error, we have estimated that there is less than a 0.11 probability of concluding that no difference in 6-keto prostaglandin F_{1α} concentrations exists between the two groups when, in fact, a difference of ≥10 ng/ml does exist. The difference in the mean values of 6-keto prostaglandin $F_{1\alpha}$ concentrations between the two groups reported by Drake et al.3 was 40.7 ng/ml.

Endometriosis group versus fertile group. Peritoneal fluid concentrations of 6-keto prostaglandin Fig. and the volumes of collected peritoneal fluid were also measured in 10 fertile women who underwent laparoscopic sterilization. The median values for total amount of prostaglandin, concentration of prostaglandin, and volume of peritoneal fluid are shown in Table I. The total amounts of 6-keto prostaglandin F_{1a} in this "fertile" group were compared with those of the "endometriosis" group by the Mann-Whitney U test (twotailed) and no difference was found ($\alpha = 0.05$). When the concentrations of 6-keto prostaglandin Fia were compared between the two groups by the U test (twotailed), there was no difference with $\alpha = 0.05$, but there

was a significant difference with $\alpha = 0.08$. The fertile group had higher peritoneal fluid concentrations of 6keto prostaglandin F_{1a} than the endometriosis group (Table I). However, this concentration difference was a reflection of the lower volumes of peritoneal fluid recovered from the fertile women when compared with the women with endometriosis (Table I) and the peritoneal fluid volumes of these two groups were significantly different ($\alpha = 0.05$) when compared by the Mann-Whitney U test (two-tailed).

Comment

The results of our study indicate that neither the amount nor the concentration of 6-keto prostaglandin $F_{1\alpha}$ in peritoneal fluid of women with endometrics was different from that in women with no inacroscopic evidence of endometriosis who belonged to infertile couples. These results conflict with those reported by Drake et al.3 who found that both the amount and the concentration of thromboxane B2 and 6-keto prostaglandin F_{10} were increased in the peritoneal fluid of women with endometriosis (n = 14) when compared to women without endometriosis (n = 15). However, the conclusions of Drake et al.3 must be considered equivocal because of several aspects of their study methodology. First, the authors did not indicate if all peritoneal fluid was sampled at either preovulatory or postovulatory time points, and second they excluded from their nonendometriosis group three women with unexplained infertility who had high levels of 6-keto prostaglandin F1a and thromboxane B2 similar to those in their endometriosis group. Although the authors suggested that microscopic endometriosis may have been present in these three women, if this disease was not visible macroscopically, it seems invalid to exclude them. A further problem with the data was the inappropriate use of the t test to analyze data that were not normally distributed.10

Although Rock et al.8 did not measure 6-keto prostaglandin F1a, they reported that the peritoneal fluid concentrations of prostaglandin E_2 , prostaglandin $F_{2\alpha}$, the metabolite of prostaglandin F, and thromboxane B₂ in women with endometriosis were not different from the concentrations in women with no evidence of endometriosis. The results of Rock et al.⁸ parallel our results with 6-keto prostaglandin $F_{1\alpha}$ levels although these investigators sampled peritoneal fluid during the proliferative phase of the menstrual cycles whereas we sampled fluid during the luteal phase for this study. In agreement with Rock et al.,⁸ we have found no difference in peritoneal fluid volumes between these two groups of women. In contrast, Drake et al.¹¹ reported that the peritoneal fluid volumes of women with endometriosis were greater than those of women without endometriosis throughout the menstrual cycle.

Comparisons of these studies under discussion are difficult for several reasons. The time points of the menstrual cycle at which peritoneal fluid was sampled either were not reported or were different for each study. Furthermore, the nonendometriosis groups of these studies were variously composed of presumably fertile women undergoing elective sterilization, infertile women with pelvic disorders and women with unexplained infertility with no evidence of a pelvic factor.

When we considered fertile women as a separate control group, we observed a higher concentration of 6-keto prostaglandin $F_{1\alpha}$ in the peritoneal fluid compared to the concentrations in women with endometriosis. However, this difference resulted from the low peritoneal fluid volumes of fertile women; when total amounts of 6-keto prostaglandin $F_{1\alpha}$ were analyzed, there was no difference between fertile women and women with endometriosis.

In no single study have all of the principal prostanoids (prostaglandin E_2 , prostaglandin $F_{2\alpha}$, 6-keto prostaglandin $F_{1\alpha}$, thromboxane B_2) been measured, but it is apparent that all of these prostanoids are present in various amounts in peritoneal fluid and it is probable that they arise from various sources. Prostaglandin: F_1 is produced by human follicles, F_2 prostaglandins F_3 and F_4 are produced by human corpora lutea, F_4 prostaglandins F_4 are produced by human endometrial stromal cells, F_4 and F_4 are formed by rabbit mesothelial cells. F_4 Keto prostaglandin F_4 was not synthesized by human endometrium in one study. We are not aware of attempts to detect 6-keto prostaglandin F_4 synthesis by human follicles or corpora lutea. The source of human

peritoneal fluid 6-keto prostaglandin F_{1a} may be the mesothelial cells of the peritoneum or it may prove to be the ovaries. Whatever the source, peritoneal fluid 6-keto prostaglandin F_{1a} levels were not elevated in women with endometriosis in this study.

We wish to thank Miss L. Thorpe for expert technical assistance.

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The partial association of uterine contractions with changes in electrocortical activity, breathing, and PaO_2 in the fetal lamb: Effects of brain stem section

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In intact fetal lambs near term there was a statistically significant relation between regular small uterine contractions and a change to high-voltage fetal electrocortical activity (excess above chance 15%) or arrest of breathing (excess 12%). Isocapnic hypoxia also arrested fetal breathing. After brain stem transection there was no relation between uterine contractions and the fetal electrocortical activity, but isocapnic hypoxia increased the rate and depth of fetal breathing. Similarly uterine contractions were to a small extent associated with the initiation of fetal breathing movements which continued for about as long as the contraction. We conclude that the occasional effects of uterine contractions are consistent with diminished fetal cranial oxygen supply. (AM J OBSTET GYNECOL 1985;152:905-10.)

Key words: Uterine contractions, fetal behavior, fetal breathing

1

In pregnant ewes, before the onset of labor, uterine contractions lasting 6 to 8 minutes occur two to three times an hour. They are therefore present about one quarter to one third of the time. They are accompanied by changes from low- to high-voltage electrocortical activity and arrest of breathing more frequently than changes in the opposite direction. The mechanisms involved may include increased fetal intracranial pressure,² compression of the fetal trunk and so stimulation of receptors in the heart, lungs, or chest wall,3 humoral messengers from the uterus or placenta, and small falls in fetal PaO, which occur during contractions.4 The cause of these falls in PaO, is uncertain; the decrease in middle uterine arterial flow during contractions was not considered large enough to influence fetal PaOo significantly.3 It is also not known whether the association of uterine contractions with arrest of breathing is direct or secondary to the change to high-voltage electrocortical activity.

We have therefore undertaken experiments to define the relationship between uterine contractions and fetal changes and to assess their biologic importance. Section of the brain stem in the upper pons in fetal lambs dissociates fetal electrocortical activity from breathing and converts the arrest of breathing during isocapnic hypoxia (in intact lambs) to an increase in frequency and amplitude. We have therefore measured the association of uterine contractions with fetal electrocortical activity and breathing movements in fetal lambs which were intact or had transected brain stems and have calculated the incidence in excess of chance. A brief account has been given elsewhere.

Methods

Seventeen Suffolk ewes (mated with Dorsets, of which all but three carried singleton fetuses) were operated on while under halothane anesthesia. Catheters were implanted in a maternal jugular vein and carotid artery, in the amniotic cavity, and in the fetal trachea, ascending aorta, and inferior vena cava. Pairs of stainless steel (Cooner Wire) electrodes were implanted into the uterus and the fetal diaphragm and onto the fetal parietal dura.5 In six fetuses the aortic catheter was a double-lumen PVC oxygen electrode (Searle 200/200) calibrated repeatedly in vivo by withdrawal of blood samples [before and during hypoxia]. In five other fetuses the cerebellum was removed by suction, and blunt transection of the upper pons was performed under direct vision just below the inferior colliculus. In other experiments removal of the cerebellum alone did not affect the fetal response to hypoxia. The advantage of the operation is that it allows transection at a defined level. A minimum of 4 days was allowed for recovery from operation. The response to isocapnic hypoxia was measured as described previously.5.7

At 121 to 134 days' gestation continuous recordings on a Schwarzer polygraph were made of the uterine

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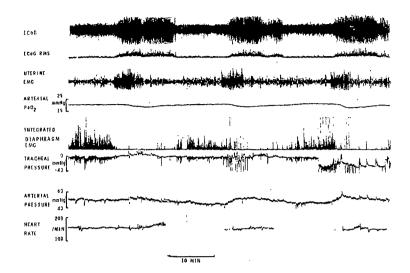


Fig. 1. Record from an intact fetal lambat 130 days' gestation, from above downward, of electrocortical activity (ECoG, raw signal and R-IS value filtered at 3 to 10 Hz), uterine electromyogram (EMG), arterial PaO₂ from a catheter-tippad electrode in the ascending aorta, integrated diaphragm EMG, tracheal pressure, arterial pressure, and heart rate. This record was selected to illustrate the association that is sometimes present between uterine contractions, the change to fetal high-voltage electrocortical activity, and arrest of breathing movements.

electromyogram; the amniotic pressure; the fetal =lectrocorticogram, raw and filtered; the diaphragm =lectromyogram; the tracheal pressure (minus amriotic pressure subtracted electronically); and (in intac fetuses) the continuous arterial Pa_{O2}. The filtered ₹MS value of the 3 to 10 Hz component (-18 db per ocave) of the electrocorticogram was continuously measured and recorded through a 6-second time-constant ategrator. This differentiates more clearly between Jowand high-voltage electrocortical activity.8 The records were analyzed visually. To avoid bias the onset and completion of episodes of uterine electromyographic activity, high-voltage electrocortical activity, and breathing and the onset of small falls in PaO, were \(\pm\)ach marked with the rest of the polygraph hidden rom view. A transparent grid was used to measure the ime in 2-minute epochs from the onset of each uterine zontraction to changes in electrocortical activity and in breathing and to falls in PaO₂ (>1 mm Hg), all occurring within the range of -4 to +16 minutes. The incidence of these events was expressed as a proportion of the number expected by chance; their significance was assessed with use of a paired t test. Altogether 14 records during 315 hours and with 816 uterine contractions were analyzed.

To examine the effect of increased pressure 16 experiments were performed on six lambs with intactmentral nervous systems. Five liters of warmed normal saline solution was infused into the amniotic cavity during 30 minutes to ensure distribution of the pressure to the whole fetus. The resting amniotic pressure did not change. A broad cuff was placed around the lower ab-

domen of the ewe and inflated to 20 or 40 mm Hg for 9 ± 0.9 minute (mean \pm SE). The mean increase in amniotic pressure was 12.4 ± 1.3 mm Hg and was similar whether the cuff was inflated to 20 or to 40 mm Hg. Maternal and fetal arterial pH, Po₂, and Pco₂, were measured before and at the end of each compression. Changes in electrocortical and breathing activity in 2-minute epochs from the onset of compression were counted.

All results are reported as means \pm SE. All the lambs studied remained in good condition throughout and after the experimental procedures.

Results

Fig. 1 shows an association between uterine contractions and a fetal change from low- to high-voltage electrocortical activity and arrest of breathing, while Fig. 2 suggests no such association. It was evident that such records, even during an hour, do not provide adequate evidence and that long-term statistical analysis was needed to establish an association. A preliminary study in two fetal lambs at 124 to 134 days' gestation (111 hours' recording with 388 contractions), with normal blood gas values, fetal breathing, and electrocortical activity, suggested that uterine activity was associated with changes in fetal behavior but that the correlation was low.

A systematic study was then undertaken in four normal fetal lambs with intact nervous systems and with blood gas values in the normal range (Table I). Breathing was associated with low-voltage electrocortical activity and was inhibited by isocapnic hypoxia. In these

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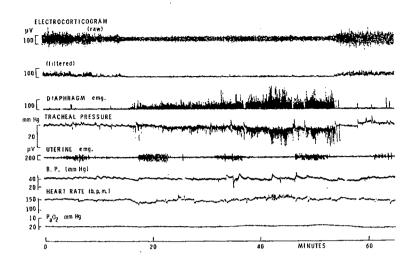


Fig. 2. Record from an intact fetal lamb at 132 days' gestation, from above downward, of electrocortical activity (raw and RMS value filtered at 3 to 10 Hz), breathing movements from diaphragm electrodes and tracheal-amniotic pressure, uterine electromyogram (emg), blood pressure, and heart rate (from electrocardiogram electrodes), and carotid PaO2. This record was chosen to illustrate a period when uterine contractions had no apparent effect upon the fetus.

Table I. Changes in blood gas values during isocapnic hypoxia in intact fetal lambs (n = 4) or after section of the brain stem (n = 5)

	F0 ₂ (mr. Hg)	Pco _z (mm Hg)	pH
Intact fetal lambs,	19.9 ± 0.7 $11.4 \pm 0.15*$	51 ± 3.7	7.30 ± 0.02
15 min of hypoxia		49 ± 3.1	7.31 ± 0.02
Brain stem sectioned,	20.0 ± 1.4	47 ± 1.4	$7.31 \pm 0.01 7.29 \pm 0.03$
15 min of hypoxia	$11.6 \pm 1.0*$	54 ± 1.5	

^{*}p < 0.001.

four lambs records with 565 uterine contractions were analyzed. The results did not differ among lambs and have been combined. The mean duration of uterine contractions was 6.4 minutes; 89% lasted from 4 to 10 minutes. At an average of three contractions an hour, contractions were present for 19.2 minutes. Hence we can calculate the incidence of changes from low- to high-voltage electrocortical activity that would coincide with contractions if the former were randomly distributed in time. The upper part of Fig. 3 shows, in the intact fetuses, the relation between uterine contractions and the change in fetal behavior, expressed as a proportion above or below that due to chance (= 1.0). During the first 2 minutes from the onset of contractions there were statistically significant (p < 0.05) peaks in the incidence of changes from low- to high-voltage electrocortical (ECoG LV-HV) activity (1.79 times greater than chance), arrest of breathing (1.95), and falls in Pa_{O₂} (2.49), which were small (1 to 4 mm Hg). The incidence of changes to high-voltage electrocortical activity and arrest of breathing fell after the first 2 minutes from the start of uterine contractions; the increase above the incidence expected by chance was con-

fined to the first 5 minutes (hatched area in Fig. 3). This composed 29% of the total time and included 44% of all changes to high-voltage electrocortical activity and 42% of arrests of breathing. There was no significant relation between uterine contractions and changes from high- to low-voltage electrocortical activity (ECoG HV-LV) or onset of fetal breathing.

The analysis in Fig. 3 is a form of signal averaging, in which the signal is the onset of a uterine contraction; the period covers 20 minutes, the approximate duration between contractions. It is obvious that a high proportion of fetal changes are not associated with contractions, that is, the correlation is low. The first 6 minutes of uterine contractions made up 29% of the total time and so should have included 29% of the changes to high-voltage electrocortical activity if randomly distributed. In fact, this time period contained an excess of 44% - 29% = 15% of such changes. By the same argument the excess of arrests of breathing associated with uterine contractions was 12%.

In the five fetal lambs that underwent transection of the brain stem uterine contractions were unaltered, with a mean duration of 6.1 minutes. Records with 251

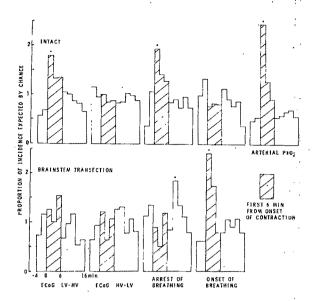


Fig. 3. Frequency distribution of changes in fetal electrocortical activity from low to high voltage (LV-HV) or vice versa (HV-LV) and of the arrest or onset of fetal breathing and falls of Pa_{O_2} in relation to the onset of uterine contractions in 2-minute epochs from -4 to +16 minutes, in four intact fetal lambs (above) and five in which the brain stem had been transected (below). The hatched areas correspond to the first 6 minutes after the onset of uterine contractions. Statistical significance (p < 0.05) where present is indicated by an asterisk.

contractions were analyzed more than 5 days after operation while breathing was still episodic; after 10 days it tends to become continuous. There was still dissociation between electrocortical activity and breathing movements (Fig. 4). There was no significant association between uterine contractions and changes in electrocortical activity (Fig. 4 and the lower part of Fig. 3). During isocapnic hypoxia (Table I) breathing continued and was enhanced as already described.⁵ The association of uterine contractions with fetal breathing movements was reversed in direction as compared with that in intact lambs. During the first 2 minutes from the onset of contractions there was a peak in the incidence of onset of breathing (2.41 times greater than chance), and after 8 to 10 minutes there was a peak in the arrest of breathing (1.84); the difference in time roughly corresponds to the duration of contractions. As in intact fetuses, the correlation between uterine contractions and changes in fetal breathing, although statistically significant, was low (Fig. 3).

It will have been noted that the changes in fetal aortic Pa_{O_2} during uterine contractions were small (mean 2.2 mm Hg). In other experiments on four intact fetuses in which the Pa_{O_2} was measured continuously we explored the effect of mild isocapnic hypoxia; the Pa_{O_2} had to be lowered by at least 6 mm Hg to cause arrest of breathing.

Uterine compression caused a 10% fall in maternal

Table II. Blood gas values before and 9.0 ± 0.9 minute after 16 episodes of maternal abdominal (and hence uterine) compression that raised amniotic fluid pressure by 12.4 ± 1.3 mm Hg in six sheep near term

,	PO ₂ (mm Hg)	Pco ₂ (mm Hg)
Control		
Ewe	106 ± 3.4	40 ± 1.8
Fetus	19.0 ± 0.9	44 ± 2.2
Compression	×	
Ewe	$95 \pm 3.5*$	38 ± 1.0
Fetus	18.3 ± 1.0	44 ± 1.9

^{*}p < 0.002.

 Pa_{O_2} while the fetal Pa_{O_2} was not significantly changed (Table II). There was no consistent effect on fetal electrocortical activity or breathing movements. During compression the number of changes in electrocortical state from low to high voltage did not exceed those from high to low voltage, nor did the incidence of cessation of breathing exceed that of its onset.

Comment

Nathanielsz et al.1 measured the incidence of fetal changes during uterine contractions. The number of changes from low- to high-voltage electrocortical activity exceeded that from high to low voltage during the 46 contractions analyzed in two fetal lambs. Various measurements of breathing indicated a preponderance of arrest over initiation of breathing during 183 to 314 contractions. We have calculated the time course of these changes in relation to the onset of uterine contractions in intact fetuses and found that the association with changes to high-voltage electrocortical activity and arrest of fetal breathing is strongest during the first 2 minutes. This corresponds with falls in fetal PaO₂ (1 to 4 mm Hg) associated with contractions, bearing in mind the relatively slow response time of the intravascular Po₂ electrode (95% in 1 minute). Such small falls in fetal Pao, do not cause arrest of fetal breathing or a change in electrocortical activity7 (this paper and unpublished observations). Partial occlusion of the uterine blood supply to decrease fetal PaO₂ by a mean of 5 mm Hg did cause a significantly increased transition from low- to high-voltage electrocortical activity in 19 of 30 trials, that is, with a low correlation.9 A greater fall in fetal Pa_{O_o} (10 mm Hg) is required to cause a consistent arrest of fetal breathing movements.7

It is agreed that a mechanism exists whereby some uterine contractions are linked with changes in fetal state. As the peak incidence of changes occurs after the onset of uterine contractions (Figs. 1 and 3) the contraction may be the primary event. Brain stem section

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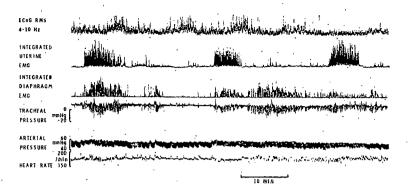


Fig. 4. Record from a fetal lamb at 124 days' gestation in which the brain stem had been transected, from above downward, of electrocortical activity (ECoG, RMS value filtered at 3 to 10 Hz), integrated uterine and diaphragm electromyogram (EMG), tracheal and arterial pressures, and heart rate. The record was chosen to show the association sometimes present between uterine contractions and the onset of fetal breathing movements. Note that there was no association between fetal electrocortical activity and either breathing or uterine contractions.

reduces the association between uterine contractions and electrocortical activity. An association between uterine contractions and breathing movements persists after brain stem section, but the effect is reversed, as is the response to hypoxia. This would be consistent with limitation of oxygen supply to the brain (and systemic arterial chemoreceptors), in part because of the small fall in Pao, and in part through a rise in intracranial pressure2 that could limit blood flow. However, we also have to take account of the fact that isocapnic hypoxia causes a substantial increase in blood flow to the medulla, pons, and midbrain in fetal lambs, both intact and after section of the brain stem.10

External compression of the maternal abdomen to cause a rise in amniotic fluid pressure by 12 mm Hg for 9 minutes on average (comparable with or greater than that produced by uterine contractions but evenly distributed because amniotic fluid volume was increased by 5 L) caused no significant change in fetal Pa_{O₀}, electrocortical activity, or breathing. Therefore, the changes induced by uterine contractions are not attributable to pressure of itself. They could be due to unevenly distributed pressure with distortion of the fetal thorax.3

An alternative proposition, that the fetus initiates the uterine contractions, seems less likely. The contractions are not altered by fetal neuromuscular blockade,11 so fetal movements do not trigger myometrial activity. We have already mentioned the fact that fetal changes appear to succeed contractions, and changes in fetal state are not always, indeed are rarely, accompanied by uterine contractions. So if a fetal hormone were released to cause both uterine contractions and a fetal change (in electrocortical activity and breathing) we must ask why the former are so regular and the latter so irregular.

We return to the biologic importance of the association between uterine contractions and fetal state changes. If uterine contractions were important to fetal development, their absence might be expected to impair development. This has not been put to the test in fetal lambs. However, in the human, extrauterine pregnancy is compatible with delivery of a normally developed infant. In the fetal lamb the correlation between regular uterine contractions before the onset of labor and irregular episodic fetal changes is low. Only 12% to 15% (changes in breathing and electrocortical activity, respectively) are associated with uterine contractions in excess of chance. These variables are therefore largely independent of uterine contractions. Their association is casual and not of primary physiologic importance.

Other observations demonstrate the independence of episodic changes in fetal electrocortical activity and breathing from the effects of uterine contractions expressed through thoracic compression or through variations in fetal PaO₂. Thus unanaesthetized mature fetal lambs still showed episodic changes in electrocortical activity, breathing, and signs of sleep states after delivery into a warm saline solution bath (the umbilical cord being intact and the ewe under epidural anaesthesia12; see also unpublished observations). These episodic changes persisted in fetuses in utero after maternal administration of nifedipine for 2 hours to diminish or abolish contractions.13 They also continued after section of the spinal cord just below the phrenic outflow¹⁴ or after section of the brain stem (see Fig. 4), the vagi, or carotid nerves. They persisted when the fetal PaO, was raised by 4 to 8 mm Hg, by giving the ewe high-oxygen mixtures to breathe7 (and unpublished observations), or to postnatal levels, by ventilation of the fetal lungs in utero.16 Therefore we conclude that these fetal

rhythms are fundamentally independent; they may rarely be entrained in association with uterine contractions by mechanisms which are not yet clear.

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CURRENT INVESTIGATION

A study of the NB/7OK and CA 125 monoclonal antibody radioimmunoassays for measuring serum antigen levels in ovarian cancer patients

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Rochester, New York, Boston, Massachusetts, and Durham, North Carolina

Serum levels of tumor antigens NB/7OK and CA 125 were determined in 101 samples by radioimmunoassays with the use of murine monoclonal antibodies. Among serum samples from ovarian cancer patients, elevated NB/7OK levels were found in 87% of samples that contained elevated CA 125 levels. With either assay, only 2% of patients with nonmalignant diseases had elevated antigen levels. No quantitative correlation was found, however, between levels of NB/7OK and CA 125. When antigen present in serum was purified by gel filtration on Sephacryl S-200, CA 125 eluted with an apparent molecular weight in excess of 100 kilodaltons whereas NB/7OK eluted with an apparent molecular weight of 70 kilodaltons. Thus the NB/7OK and CA 125 radioimmunoassays appear to measure two different populations of antigenic material. (AM J OBSTET GYNECOL 1985;152:911-13.)

Key words: Monoclonal antibody, ovarian cancer, radioimmunoassay

Tumor-associated antigens may prove useful as markers for the detection and monitoring of ovarian cancer. Two such antigens, namely, NB/7OK¹ and CA 125,² have been previously described by our laboratories. NB/7OK appears to be present in most major types of epithelial ovarian cancer, while CA 125 appears to be a marker for nonmucinous ovarian tumors. Levels of both antigens appear to be elevated in sera from patients with ovarian cancer when compared to sera from patients with benign nontumorous gynecologic disease. Monoclonal antibodies have been prepared against each of these antigens and radioimmunoassays have been developed for the measurement of serum antigen levels³ (Knauf S, et al., submitted for publication).

In this communication, we report a study of NB/7OK and CA 125 tumor antigen levels in the sera of ovarian cancer patients and control patients with nontumorous gynecologic disease and demonstrate that the radio-

immunoassays for these two tumor antigens measure different antigenic populations.

Material and methods

Serum samples. Blood samples were obtained from 41 patients with benign nontumorous gynecologic diseases and from 60 patients with confirmed ovarian cancer. Serum harvested from these samples was stored at -70° C. Samples for testing in the NB/7OK assay were selected by R. C. B. to provide CA 125 values in excess of 35 U/ml.

Chromatography of serum on Sephacryl S-200. Pools of sera were prepared from five normal healthy control subjects and from six ovarian cancer patients. Aliquots of 3 ml from each pool were dialyzed for 4 to 16 hours at 4° C against phosphate-buffered saline. Each dialyzed serum pool was applied separately to a 1.6 by 64 cm column containing Sephacryl S-200 equilibrated in phosphate-buffered saline at room temperature. Approximately 0.8 ml fractions were collected at a flow rate of about 26 ml/hr. Fractions were examined for antigenic activity by radioimmunoassay and for protein content by absorbance measured at 280 nm (OD₂₆₀).

Assays for measuring antigenic activity. Antigenic activity was measured by radioimmunoassays for NB/7OK (Knauf S, et al., submitted for publication) and

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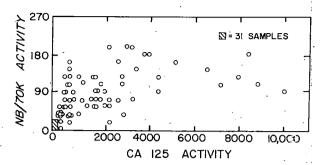


Fig. 1. NB/7OK and CA 125 activity of 101 serum samples from ovarian cancer patients and control subjects as measured by radioimmunoassays with the use of murine monocimal antibodies. NB/7OK activity is expressed per sample assured (5 μl) and CA 125 activity is expressed per milliliter of undiluted serum.

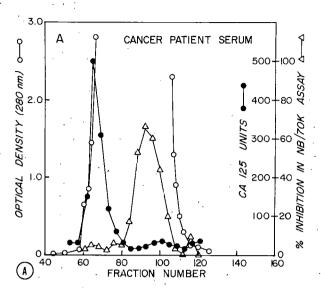
Table I. NB/70K and CA 125 levels in 101 serum samples as measured by radioimmunoassay*

	. Assay				
Sample	NB/70K	CA 125			
Nonmalignant gynecologic					
disease $(n = 41)$	•,				
Range	0-53	1-37			
Mean	14	8			
No. and %, of samples with elevated antigen levels	1 (2%)	1 (2%)			
Ovarian cancer $(n = 60)$	•				
Range	37-200	162-10,0汽			
Mean ·	. 87	2345			
No. and % of samples with elevated antigen levels	53 (87%)	60 (100 <i>%</i> -			

*Serum samples were assayed by radioimmunoassays —ith the use of murine monoclonal antibodies as described in *faterial and methods. Antigenic activity is expressed in tern= of arbitrary activity units relative to reference standards. Act vity is given per sample assayed in the case of NB/70K and per milliliter of undiluted serum in the case of CA 125. The paramal cutoff values used were 45 and 34 units for the NB/DK and CA 125 assays, respectively.

CA 125³ as previously described. Both assays use □urine monoclonal antibodies and express antigenic □ctivity in terms of arbitrary units of activity relative □ a reference standard. For serum samples, the equivalent of 5 and 100 µl of undiluted sample was assayed in □he NB/7OK and CA 125 assays, respectively. For Sepha □yl S-200 column eluates, the equivalent of 10 or 10C µl of every fourth column fraction was assayed for □B/3 7OK and CA 125 activity, respectively. In all cases, □ctivity was expressed per sample in the case of the □B/3 7OK assay and per milliliter of undiluted sample in □he case of the CA 125 assay.

Statistical evaluation. Regression analysis was used to investigate the correlation of serum antigen le-sls



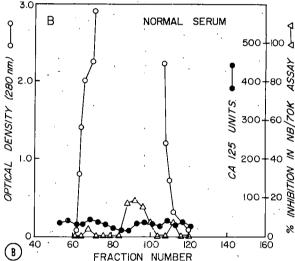


Fig. 2. Sephacryl S-200 chromatography of (A) a pool of serum from patients with confirmed ovarian cancer and (B) a pool of serum from normal healthy control subjects. Fractions were assessed for total protein content by OD_{280} and for antigenic activity by radioimmunoassay.

measured by the two radioimmunoassays. For samples with an NB/7OK activity level greater than 200 units per sample, the value of 200 was used for statistical analysis.

Results

Serum NB/7OK and CA 125 antigen levels were determined in 101 serum samples. The results are summarized in Table I and Fig. 1. Not only did the range of antigen values differ in the two assays, but the rank order of samples was also significantly different. In each assay a single sample from a patient with non-malignant gynecologic disease was positive. The patient with elevated serum NB/7OK levels, however, was not

the same patient with elevated serum levels of CA 125. Furthermore, analysis of NB/7OK and CA 125 levels for the samples by linear regression indicated a value for r² of 36%, suggesting that the two assays measure different antigens.

Pools of sera from normal healthy control subjects and from ovarian cancer patients were fractioned on Sephacryl S-200 and the resulting column eluate was examined for antigenic activity by radioimmunoassay. As can be seen in Fig. 2, CA 125 activity eluted in the void volume, indicating a molecular weight in excess of 105 daltons. By contrast, NB/7OK activity was not excluded from the gel and eluted with a molecular weight similar to that of bovine serum albumin.

Comment

The results presented here indicate that the monoclonal antibody assays for NB/7OK and CA 125 measure different antigens in serum and that the level of either of these antigens is independent of the other. Both assays appear to have specificity for ovarian cancer in the serum samples examined. Both assays also have a very low rate of false positivity among patients with benign, nontumorous gynecologic disease (data presented here) and also among apparently disease-free control subjects3 (Knauf S, et al., submitted for pub-

The serum samples from ovarian cancer patients ex-

amined in this study were selected by R. C. B. for CA 125 positivity. Of these samples, all but seven also had elevated NB/7OK serum levels. Since CA 125 appears to be a marker for nonmucinous ovarian tumors, the samples in the present study did not include samples from patients with mucinous ovarian cancers. Since the NB/7OK assay is capable of detecting tumor antigen in this type of patient, it is possible that (1) the overall positivity of the NB/7OK and CA 125 assays may be similar if all types of ovarian cancers are considered and/or (2) a combination of the two assays may be useful for detecting most major pathologic types of ovarian cancers. Studies to examine these possibilities are currently in progress.

We wish to acknowledge the assistance of Ms. J. Kalwas, Ms. J. Huff, Ms. E. Schaetzl, Ms. A. Battaile, and Ms. Ann Rhinehardt.

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Uterine rupture and labor induction with prostaglandin

To the Editors:

Having read the reports on two cases of rupture of the unscarred uterus following attempts at induction of labor with vaginal prostaglandin reported by Claman et al. (AM J OBSTET GYNECOL 1984; 150:889), I wish to draw attention to the presence of a number of specific risk factors which apparently were overlooked or introduced. First, the dose of prostaglandin administe-ed was much too large: vaginal dosages of prostaglandin E₂ administered to parous women for both preinduction (cervical softening) and induction of labor should not be higher than 5.0 mg whatever the medium (suppository, tablet, gel) used. Second, in both patients prostaglandin treatment was initiated while the myometrium was still under the influence of oxytocin, which explains the potentiated uterotonic effect of prostaglandin. Finally, reservations can be made regarding the application of potent oxytocic drugs to grand multiparous women, and as already mentioned, it is definitely asking for trouble to administer 10 mU/mir of oxytocin and 20 mg of intravaginal prostaglandin Ξ_2 sequentially.

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Neurofibromatosis: Cause or coincidence of pregnancy complications?

To the Editors:

The communication by Belton et al. (AM J OBSTLT GYNECOL 1984;149:468) describes a woman with neurofibromatosis "complicated" by intrauterine growh retardation and oligohydramnios. A more probable interpretation of the case would be that poor nutrition, in part related to hyperemesis, led to inadequate weight gain and placental hypoperfusion, perhaps further ciminished by the hypertension, in a woman who happened to have neurofibromatosis; these events would lead to intrauterine growth retardation, which is frequently accompanied by oligohydramnios, and are so common that the rarity of the patient's disease should not obscure the much more likely explanation of her problem.

A pity that the placenta was not examined.

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REFERENCE -

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Reply

To the Editors:

Our report concerning neurofibromatosis in pregnancy was intended to alert clinicians to the possibility of underlying vascular involvement and its potential adverse sequelae. Greene et al.1 have categorized vascular involvement according to the diameter of the vessel: "large" vessels (>1 mm) demonstrate intimal hypertrophy, fragmentation of the media and elastic laminas, a fibrous adventitial reaction and may be surrounded by neurofibromatous tissue. "Smaller" vessels (<1 mm) demonstrate dysplasia of the vessel wall which may consist of a pure intimal form, an intimal-aneurysmal form, or a periarteriolar nodular form. These changes may occur in any arterial vascular bed in the body, including the uterus. Although the incidence of these changes in the vessels in neurofibromatosis is not known, they may be common.2 Swapp and Main2 postulate that hypertension (a well-known risk factor for intrauterine growth retardation) may result when affected vessels are unable to expand when challenged by the increased blood volume during pregnancy. Under these circumstances one would expect to find "significant" hypertension in association with intrauterine growth retardation. We are convinced, however, that intrauterine growth retardation may occur soley on the basis of vascular involvement affecting uteroplacental perfusion independent of the degree of hypertension. Further, it is not difficult to imagine that these vascular lesions may proliferate because of the influence of pregnancy, as has been commonly noted with the cutaneous lesions of neurofibromatosis.2 The degree of intrauterine growth retardation would thus not necessarily reflect the degree of hypertension seen in the clinical setting. Because we cannot determine which pregnancies complicated by neurofibromatosis have vascular involvement that may impair uteroplacental perfusion, we recommend routine serial ultrasound examinations to screen for intrauterine growth retardation in this group of patients. In addition to intrauterine growth retardation, vascular involvement in neurofibromatosis may threaten maternal well-being. Brady and Bolan³ have recently reported on a maternal death caused by a dissecting aneurysm associated with vascular neurofibromatosis. Clearly, pregnant women with neurofibromatosis should be considered high-risk obstetric

We would like to reiterate one of our summary statements: "The maternal weight gain was poor and there was coexistent mild hypertension, but these factors

alone, in our experience, seldom result in growth retardation of the magnitude observed. Certainly, the possibility of vascular involvement of the uterine circulation (as described by Gleicher et al.4) resulting in reduced uteroplacental perfusion and in diminished fetal growth cannot be overlooked."

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Umbilical artery waveform?

To the Editors:

Fleischer et al. (Umbilical artery velocity waveforms and intrauterine growth retardation. Am J Obstet Gynecol 1985:151:502) report their continuing experience with continuous-wave Doppler measurement of peak-systolic/diastolic ratio of the umbilical artery waveform in normal and growth-retarded gestations. A normal range for gestational age of this ratio is described.

I am concerned with the certainty with which vessel identification is made. In the material and methods section of this article and its parent article, the Doppler transducer (without the concurrent use of linear-array real-time ultrasound) identifies the umbilical artery by the characteristics of its waveform. The transducer has a fixed effective depth of 8 cm. In the earlier article, it is stated that "the fetal aorta waveform is easily distinguished from the umbilical artery flow pattern because of its triangular shape and lack of diastolic flow, as opposed to the umbilical arterial flow, which has an exponential decay from systole and a tapering diastolic component."

I disagree with Fleischer et al. with regard to this differentiation. Previous reports using concurrent linear-array and Doppler ultrasound with variable depth gating (that clearly identify sampling to be from the fetal aorta) have demonstrated the tapered diastolic component to be a normal part of the waveform of the fetal aorta. Altertions of diastolic waveform in the

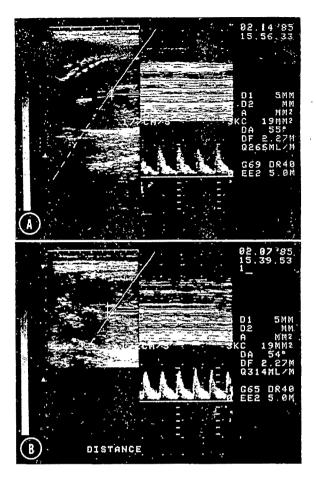


Fig. 1. Fetal aortic waveform in two patients (A and B). Note sampling is clearly from the fetal aorta in each case.

fetal aorta have been described2,3 which are similar to those reported by Fleischer et al. and attributed to the umbilical artery. In our own ultrasound unit we have examined the fetal aortic waveform in 50 patients (in an ongoing study) using pulsed Doppler fixed at a 55 degree angle to a linear-array transducer. More often than not, a waveform is obtained that has a diastolic component similar to, if not identical to, that ascribed to the umbilical artery by Fleischer et al. in their blind technique (Fig. 1). Is it possible that what Fleischer et al. have described as waveform from the umbilical artery is at times, if not frequently, produced by the fetal aorta? Would not the concurrent use of linear-array ultrasound in at least a portion of their patients be necessary to demonstrate that the waveform measured is consistently from the umbilical artery?

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Reply

To the Editors:

We appreciate that Dr. Spinnato commented on <code>purrecent</code> publication on umbilical artery velocity wereforms. Most investigators now believe that there <code>a</code> a definable diastolic velocity component in the descending aorta of the average—for—gestational age fetus. We would not disagree with this contention; however, the diastolic signal is frequently quite noisy or turbul—nt, hence we had decided not to use it as a useful site—for fetal evaluation. This concern seems to have been <code>r=inforced</code> by the reports of normal aortic flow. For example in Griffin et al.¹ normal rates of 246 ml/kg/min <code>prereceported</code>, but others suggest values averaging 16½ to 216 ml/kg/min.²

Dr. Spinnato's illustrations highlight how disag-eements may arise. As he mentioned, their equipment differs from ours. The spectral analysis may differ, and the time-frame freeze appears to be different. Furthermore, a fetal tachycardia may also influence the diastolic component. Hence comparisons are problematic.

We have had the opportunity of studying more tan a thousand fetuses and are convinced that our hyperhesis is reasonable; that is, in most circumstances 1 is easy to differentiate aortic signals from umbilical. Fortic systotic/diastolic ratios are normally around 7 \sim 8 whereas umbilical ratios are ~2.5 to 3 and rarely >6. The advantage of a continuous-wave system without linear-array imaging is that repetitive studies can be carried out without exceeding current established safety guidelines and that a powerful diagnostic to-d is available at the bedside or in the office rather than in an expensive laboratory.

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Cicatricial pemphigoid of the vulva

To the Editors:

I was interested to read the report "Bullous pemphigoid of the vulva: A case report" by Stage et al. Am J OBSTET GYNECOL 1984;150:169).

The fact that the patient had lesions on the scalp and in the mouth and pharynx makes it likely that she had cicatricial pemphigoid rather than bullous pemphigoid. The latter condition very rarely affects mucosal surfaces whereas such involvement and also lesions of the scalp and vulva are common in the cicatricial type.

It may therefore be highly relevant that the patient was taking a β -blocking agent since, as the authors point out, there is a previous reference in the literature to the particular relationship of a β -blocker to anogenital cicatricial pemphigoid.

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Reply

To the Editors:

Dr. Ridley's comments on our report "Bullous pemphigoid of the vulva" are indeed pertinent and appreciated. The format of this presentation required brevity and did not allow for any discussion of bullous diseases in depth.

Bullous pemphigoid is a chronic, benign, vesicular disease of the skin and mucous membranes. Lesions of the vulva may occur, but vaginal lesions are rare. The bullae are subepidermal, and a linear deposit of IgG, IgM, and/or complement can be demonstrated by immunofluorescent studies along the basement membrane zone in 90% of patients. In addition to subepidermal bullae, dense eosinophilic and perivascular lymphohistiocytic infiltration of the dermis is noted microscopically. Benign mucous membrane pemphigoid, or cicatricial pemphigoid, is a variant of bullous pemphigoid involving mainly the mucous membranes including the buccal membranes, nasal mucosa, larynx, esophagus, genitalia, and anal area. Skin lesions occur in only 10% to 30% of such patients. Chronic erosions of the mucous membranes in benign mucous membrane pemphigoid can often lead to symblepharon of the eye and blindness, and adhesions and strictures of the larynx and esophagus as well as in the vagina. Benign mucous membrane pemphigoid tends to run a more chronic course and is at times less responsive to therapy. Circulating antibodies to the basement membrane zone antigens are present in 90% of patients with bullous pemphigoid, but contrary to what was previously thought, differentiation between bullous pemphigoid and benign mucous membrane pemphigoid cannot be made by the presence or absence of circulating antibodies.1 In one study, mucous membrane involvement was especially noted in patients with bullous pemphigoid when indirect immunofluorescent studies were negative.2 The pathologic picture is identical in both, but there is a lower incidence of circulating antibodies in benign mucous membrane pemphigoid as well as a tendency to ocular involvement, slow healing, and scar formation. No consistent laboratory data except biopsies and immunofluorescent studies are helpful in making the diagnosis.

In trying to satisfactorily answer Dr. Ridley's question as to whether the case reported represents bullous pemphigoid or benign mucous membrane pemphigoid (cicatricial pemphigoid) the following features were evaluated: (1) The perineal lesion was felt to have originated on the perineal skin and extended to the mucosa of the labia minora. The vagina was not involved. (2) The lesions healed rather promptly with steroid treatment, and there was no scarring. (3) There was no eye involvement. (4) Although the indirect immunofluorescent studies on circulating antibodies were negative, this is in accordance with the reported greater involvement of mucous membranes involvement when indirect immunofluorescent studies are negative.

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Assessing risks of midtrimester amniocentesis

To the Editors:

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I was pleased to see the recent publication by Crane and Kopta, entitled "Genetic amniocentesis: Impact of placental position upon the risk of pregnancy loss" (AM J OBSTET GYNECOL 1984;150:813). They are to be congratulated on their excellent outcome record, and I would strongly support their suggestion that the use of real-time sonographic guidance, small gauge needles, and efforts to avoid the central portion of anterior placentas are important in achieving such an excellent record following midtrimester amniocentesis.

They concluded that transplacental amniocentesis is associated with little if any increased risk of reproductive loss. Unfortunately, the data presented in this current manuscript do not support that conclusion for the reasons outlined in Table I. The three groups (A, B, and C) assume that the "true" rate of loss in amniocentesis is between 0.1% and 1% depending on whose data one chooses to believe and what one's biases are. If one hypothetically presumes that traversing the pla-

centa doubles the "true" rate of loss as we suggested in our 1982 publication (AM J OBSTET GYNECOL 1982;143:653), you will see by the table that the numbers of patients in the groups presented in Dr. Crane's work are not large enough to detect such a minor increase in risk. For example, if a true rate of loss is 0.5% and traversing the placenta doubles that risk to 1%, then at a 5% significance level with use of a two-tailed Fisher's exact test, the current study group sizes would only have a 10% chance of detecting such a change (0.099). Probably closer to the truth is the difference between 0.1% and 0.2%, wherein the current study group sizes would only have a 0.3% chance (0.003) of detecting such a change at a 5% significance level.

As the authors noted in their manuscript, occasionally one can observe bleeding from the chorionic plate under ultrasound following amniocentesis in which the needle has traversed the placenta. My bias would be that if one does that enough times eventually one will exsanguinate a midtrimester fetus. Since the prevalence rate of these problems is so small in experienced hands, detecting a potential doubling of this risk requires exceedingly large study groups. We encountered this difficulty even in our much larger study, since we were unable to show a significant difference in those losses which occurred within 7 days. As Dr. Crane noted, we had to invoke the entire number of losses at any time following amniocentesis to reach a 0.05 significance between posterior and anterior placentation.

As I indicated in a earlier correspondence (AM J OB-STET GYNECOL 1983;146:345), these issues are of more than academic interest. Increasing numbers of patients whose circumstances do not suggest an especial increase in abnormal findings are requesting prenatal diagnosis. Although obtaining this information is a relatively safe procedure, one would be remiss not to accurately counsel such patients that the risk of compromising what is highly likely to be a normal pregnancy may be increased in circumstances that require the needle to pass through a large portion of placenta.

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Reply

To the Editors:

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We appreciate Dr. Porreco's analysis of our data and the opportunity to respond to his letter. We agree that

Table I. Assumed rates of reproductive loss (%)

Group	Nontraversed placenta $(n = 651)$	Traversed placenta (n = 347)	One-tailed test	Two-tailed test
A	1.0	2	0.273	0.198
В	0.5	1.0	0.116	0.099
C	0.1	0.2	0.018	0.003

Fishers' exact power to detect, p = 0.05.

Table I. Pregnancy outcomes in 3001 women undergoing genetic amniocentesis

. ·	1	rans- cental	Nontrans- placental		
Pregnancy outcome	n.	%	n	%	
No. of women	918	30.6	2083	69.4	
Spontaneous abortion within 1 week of tap	. 0		1	•	
Spontaneous abortion <24 weeks' gestation	6	0.65	20	0.9€	
Intraamniotic bleeding observed after tap	95	10.4	0.		

Table II. Statistical power to detect increased risk (if present) of traversing the placenta during genetic amniocentesis based on 3001 cases with known pregnancy outcomes (918 transplacental taps)

Theoretical difference	One-tailed test	Two-tailed test
1.0% vs. 2.0% risk	0.58759	0.46265
0.5% vs. 1.0% risk	0.37064	0.25938
0.1% vs. 0.2% risk	0.14488	0.08476

the study population (n=998) is too small to confidently exclude a minor but statistically significant increase in the risk of pregnancy loss with transplacental amniocentesis. It is important, however, to distinguish between "statistical" and "clinical" significance. The major conclusion of our paper is that transplacental genetic amniocentesis is associated with minimal risk when performed with a small-gauge needle and real-time ultrasound guidance.

We now have delivery outcome data on 3001 genetic amniocentesis patients, including 918 transplacental taps. The overall incidence of subsequent pregnancy loss (Table I) is only 0.87% (0.65% among transplacental taps versus 0.96% when placenta is not traversec). No reproductive losses have occurred in the 95 women in whom intraamniotic bleeding from the chorionic plate was observed after amniocentesis.

Despite larger numbers, the statistical power of our data remains low (Table II). With use of a two-tailed Fisher's exact test, the enlarged data base now allows a 46% chance of detecting a doubling in the risk of pregnancy loss from 1.0% to 2.0% following transplacental amniocentesis. The difference between a 0.5% and a 1.0% risk would have a 26% chance of being detected.

Although our sample size now exceeds that in Γ r. Porreco's study (2219 cases), we are unable to confirm his finding of a twofold increase in pregnancy loss with anterior placentation (3.06% versus 1.57% with posterior placenta). We believe this difference is attributable to our use of a smaller-diameter needle (22 gauge) and real-time sonographic guidance during needle in-

sertion. This technique allows selection of the thinnest area of placenta, preferably near its margin and away from the cord insertion.

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Gestational diabetes and birth trauma

To the Editors:

We have read the paper of Coustan and Imarah (Prophylactic insulin treatment of gestational diabetes reduces the incidence of macrosomia, operative delivery, and birth trauma. Am J OBSTET GYNECOL 1984; 150:836-42) with some concern, since it suggests that gestational diabetes is associated with an increased incidence of birth trauma which can be reduced by maternal administration of insulin. Before further interventions of unproved benefit are associated with this doubtful diagnosis, we would like to take issue with some of the points raised by this paper.

First, the study is nonrandomized. As a result 72% of patients treated with insulin were cared for by the university service, whereas 80% of patients who received no treatment were delivered by the private service. Although the authors reject the possibility, it is clearly possible that a conservative philosophy in the management of the second stage of labor and reluctance to use midforceps in the university service could account for the same findings.

Great differences in the incidence of birth trauma between centers (and services) do exist. A recent article from a large hospital in Montreal reports a 1.8 incidence of birth injury (peripheral nerve or bony fracture). In our city for the years 1982 and 1983, there were 33 cases of this type of birth injury from 12,848 deliveries (0.26%). In one hospital, however, during that time there were no recorded cases in 4076 deliveries, whereas in the other two community hospitals there were 24 and nine cases respectively (from 4755 and 4017 deliveries respectively).

The second point is that birth trauma is not necessarily confined to macrosomic infants. Gabbe et al.² noted a 2% incidence of birth injury (as defined above) in a series of patients with gestational diabetes who were delivered of normal-sized infants. Of the 33 cases in Hamilton, only 11 infants weighed more than 4000 gm. One might extrapolate that if it was possible to avoid the development of macrosomia in all pregnancies, the number of birth injuries would only be reduced by one third.

It would be interesting to know what percentage in Coustan and Imarah's report weighed in fact less than 4000 gm. Furthermore, the background incidence of birth trauma in the various services in their hospital

would be of interest; one suspects the same gradient would exist irrespective of the presence or absence of glucose intolerance.

Significant reduction in birth trauma is more likely to be accomplished by a change in philosophy of labor management rather than by institution of any program of screening for glucose intolerance and the use of insulin. It is likely that the conclusions from Dr. Coustan's earlier randomized control trial of insulin therapy in "gestational diabetes" which showed no difference in operative delivery or birth trauma is likely to be nearer the truth despite its small sample size.³

We feel gestational diabetes is a diagnosis still looking for a disease. This paper fails to establish either that making the diagnosis or administering insulin to the patients with impaired glucose tolerance in pregnancy improves the perinatal outcome.

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Reply

To the Editors:

I am delighted that Dr. Hunter and Ms. Milner found our article interesting enough to merit comment and am grateful for the opportunity to respond.

These writers suggest that the differences in distribution between private and university service among the three treatment groups might have accounted for the differences in mode of delivery. We mentioned in the original paper that this seemed unlikely to us. However, in response to their suggestion we have reviewed the monthly obstetric statistics from the Yale—New Haven Medical Center during the period studied (July 1, 1975, through July 1, 1980). During these five years

23,428 infants were delivered. On the university service, operative deliveries (primary cesarean sections, midforceps deliveries, and vacuum extractions) accounted for 19.2% of the births, while on the private service 18.4% of infants were delivered operatively. To make these statistics comparable to those described in our paper, we eliminated births by repeat cesarean section and breech vaginal deliveries from the denominators. When this correction is made, the statistics remain similar. On the university service 20.9%, and on the private service 19.7%, were delivered operatively. I believe these statistics strengthen our argument that the source of care was not a significant confounding variable, since Hunter and Milner's "null hypothesis' would require that there be a higher incidence of operative delivery on the private service than on the university service.

I believe it is a reasonable assumption that birth trauma is not necessarily confined to macrosomic infants, as we traditionally define macrosomia. The use of some arbitrary cutoff for macrosomia does not relate the particular infant's size with the maternal pelvis. For example, a woman with a small pelvis might have difficulty delivering an infant who weighs 3000 gm and might sustain birth trauma, yet we would not define that infant as being macrosomic. Therefore, I do not believe it is relevant to ask whether birth trauma was confined to infants weighing more than 4000 gm; in fact, it was not.

The last paragraph of their letter leaves me somewhat mystified. Although the strength of the association between gestational diabetes and other adverse outcomes is the subject of current debate, there is ample evidence that gestational diabetes is associated with an increased incidence of macrosomia, however one might define it. It seems to me untenable to assert that relative birth weight has nothing to do with difficult or traumatic delivery. At least two randomized trials have shown that prophylactic insulin therapy reduces the incidence of macrosomia. Therefore, our paper serves as a link between the lowering of macrosomia on the one hand and the anticipated lowering of operative delivery and birth trauma on the other.

Donald R. Coustan, M.D.

Department of Obstetrics and Gynecology Women & Infants Hospital of Rhode Island 50 Maude Street Providence, Rhode Island 02908 Announcements of major meetings and other significant activities must be received at least 8 weeks before the desired month of publication. All announcements carry a charge of \$45.00 U.S. and the fee must accompany the request to publish. Information will be limited to title of meeting, date, place, and an address to obtain further information. Send announcements and payment, parable to this JOURNAL, to The C. V. Mosby Company, 11830 Westline Industrial Drive, St. Louis, Missouri 63146.

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- Intensive Board Review Course in Reproductive Endocrinology, September 13-16, 1985, Massachusetts General Hospital, Boston, Massachusetts. Contact: Dr. William F. Crowley, Jr., Vincent Research Laboratories, Massachusetts General Hospital, Boston, MA 02114.
- London and Paris Fall Ultrasound—Obstetrics and Gynecology, London, England, September 20-25, 1985; Paris, France, September 25-29, 1985. For further information contact: Secretary, Fall Ultrasound Symposia, Medical Seminars International, West Park Medical Office Building, 22135 Roscoe Blvd., #104, Canoga Park, CA 91304. Tel.: (818) 340-0530, ext. 280.
- Symposium on the Prevention of Osteoporosis. September 29-October 2, 1985. Westin Ilikai Hotel, Honolulu, Hawaii. For further information contact: Dr. Philip Ross, Associate Director, Osteoporosis Center, Kuakini Medical Center, 347 North Kuakini Street, Honolulu, Hawaii 96817. Tel.: (808) 547-9578.

- Conference on Smoking and Reproductive Health,
 October 15-17, 1985, San Francisco, California. Sponsored by Family Health International and other organizations. For further information contact: Michael Rosenberg, M.D. M.P.H., Family Health International, Research Triangle Park, NC 27709. Tel.: (919)
- Specialty Review in Obstetrics and Gynecology, October 28–November 2, 1985, Chicago, Illinois. For further information contact: Dick Nelson, Course Manager, The Cook County Graduate School of Medicine, 707 South Wood St., Chicago, IL 60612. Tel.: (312) 633-2600.
- Behavioral Obstetrics and Gynecology. November 1-2, 1985. Towsley Center, Ann Arbor, Michigan. For further information contact: Betty Phillips, The Office of Continuing Medical Education, The Towsley Center, Box 057, The University of Michigan Medical School, Ann Arbor, MI 48109-0010. Tel.: (313) 763-1400.
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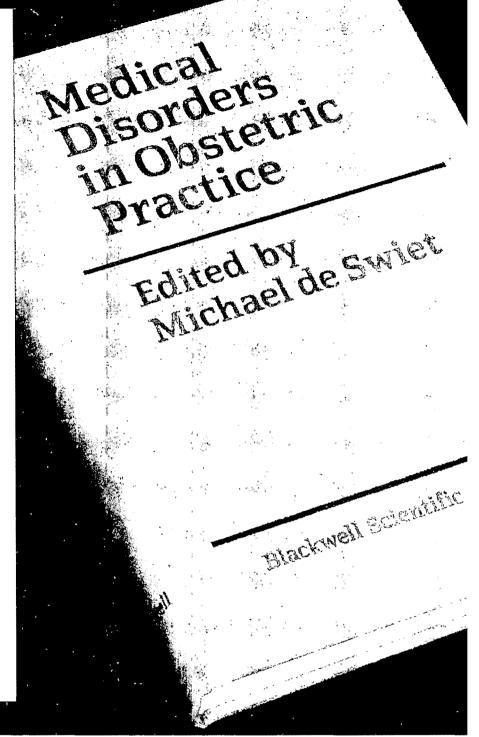
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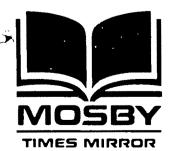
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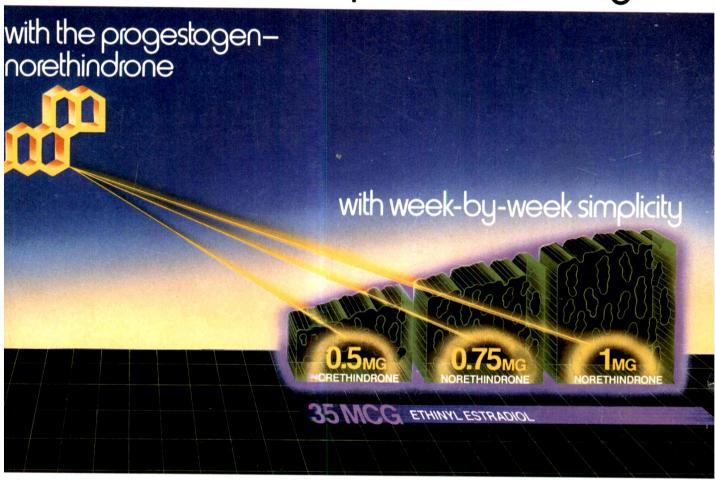
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INTERNATIONAL SYMPOSIUM ON VULVOVAGINAL MYCOSES

June 1-3, 1984 Fort Lauderdale, Florida

Guest Editor RICHARD L. SWEET, M.D.

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INTERNATIONAL SYMPOSIUM ON VULVOVAGINAL MYCOSES

Importance of differential diagnosis in acute vaginitis

Richard L. Sweet, M.D.

San Francisco, California

Acute vaginitis is one of the most common diseases seen in the practice of office gynecology. Large survey studies of women with lower genital tract symptoms suggestive of vaginitis have demonstrated the presence of three major etiologic categories in acute vaginitis: (1) nonspecific vaginosis (Gardnerella vaginalis), (2) vulvovaginal candidiasis (Candida albicans), and (3) trichomoniasis (Trichomonas vaginalis). Effective treatment of acute vaginitis requires that an accurate diagnosis be established and etiologic microorganism(s) be identified. In general, the differential diagnosis of acute vaginitis does not rely on elaborate technology but, rather, requires inexpensive and readily available office equipment and supplies, a detailed history, and an adequate examination of the external genitalia, vagina, and cervix. Only after the etiology of vaginitis has been identified can appropriate therapeutic intervention(s) be utilized. (AM J OBSTET GYNECOL 1985;152:921.)

Key words: Vaginitis, differential diagnosis, nonspecific vaginosis, vulvovaginal candidiasis, trichomoniasis

Effective treatment of acute vaginitis relies on making an accurate diagnosis and identifying the causative microorganisms.

Some of the reasons for failures in the treatment of vulvovaginitis relate to inaccurate, inadequate diagnosis—sometimes a diagnosis is based only on the type of discharge the patient describes. At times, physicians even diagnose the disease over the telephone and call a prescription in to the local pharmacy. In addition, the use of a broad-spectrum, "shotgun" approach often results in failure. Finally, some of these cases may be sexually transmitted, and the sexual partner will have to be treated to prevent recurrences.

Some physicians treat on the basis of only a Papanicolaou smear. There is sometimes a failure to use the treatment of choice and a failure to use what Dr. James Ingram, of the University of South Florida, calls the "dry vulva regimen" (personal communication, 1983), which is an attempt to circumvent the common practice of the wearing of pantyhose and synthetic clothing, which aggravates vaginitis.

Women who have complaints of lower genital tract symptoms, such as discharge, urinary frequency or dysuria, abnormal bleeding, itching, and/or foul odor, have disease entities that basically fall into four major categories. The first two are cystitis and urethritis, with causative organisms such as Neisseria gonorrhoeae, Chlamydia trachomatis, and herpes simplex virus. The emphasis in this presentation, however, is on inflammation of the vulva and vagina, exclusive of herpes. The three major causes of vulvovaginitis are Trichomonas vaginalis, Candida albicans, and Gardnerella vaginalis.

On approaching the patient with symptoms of lower genital tract infection, there is a basic, systematic format. It is relatively easy to use, but unless the steps are compulsively followed, major omissions can occur.

As far as history taking goes, it is important to focus not only on the patient's description of discharge but also on concomitant findings. Is pruritus present? Is there odor? Are urinary tract symptoms present? Are there lesions on the vulva? Different symptoms will lead the physician to different diagnostic categories.

During examination it is important to closely evaluate the cervix and the vagina and to evaluate the discharge microscopically as well as grossly.

Simple equipment that is readily available in most offices, clinics, and hospital emergency rooms facilitates differential diagnosis in a patient with acute vaginitis. The diagnosis does not depend on elaborate technology but, simply, a microscope, some glass slides and covers, saline solution preparations, potassium hydroxide,

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Gram stain, pH paper, the Tzanck test (which is very similar to a Papanicolaou smear), and possibly some culture media for *Candida*.

We culture for *Gonococcus* and *Chlamydia* routinely but do not routinely culture for yeast, *Gardnerella* and *Trichomonas*.

Another area that is crucial, especially in patients with recurrent vaginitis, is that the sexual partner needs to be examined as well. Furthermore, although the etiologic agent may not be found in the woman, close examination of the sexual partner may sometimes bring results.

The physiologic state of the vagina during the female life cycle may have major impact and may explain some of the symptoms, especially as they relate to the menstrual cycle and to periods in a woman's life. At birth the newborn baby actually reflects, in the vaginal environment tends to have an alkaline pH. There tends to be some moderate thickness in the vaginal epithelium, and glycogen is present as the result of maternal estrogen. There are very few lactobacilli present, but there is a fair amount of protection for the newborn infant from vaginal infections as a result of the effects of maternal estrogen and amniotic fluid.

About day 4, the maternal estrogen effect has its maximum approach. The amniotic fluid is gone. A fairly acidic pH begins to develop, and some lactobacilli are present. As a result, there is some moderate protection against infection. Once the maternal estrogen is gone, by about the first or third week of the infant's life, the situation is that of the prepubertal female, in which there are very few lactobacilli and very little estrogen is present. Thus the young woman has minimal thickness of the vagina, minimal glycogen, and an alkaline pH. As a result, she has only minimal protection against vaginal infection.

Once young women go through puberty, they begin to produce estrogen again, with resultant thickening of the vaginal epithelium and with glycogen and lots of lactobacilli present. The pH begins to decrease again, and once more they have fairly good protection against vaginal infections.

Pregnancy is the optimum situation and maximizes protection in the vagina against infection, with the exception of *Candida*. It is at this point that women have the most acidic pH, multitudes of lactobacilli, the thickest vaginal epithelium, and the highest glycogen content. The high glycogen and estrogen levels are what contribute to the growth of *Candida*.

Then the cycle completes its course, and the postmenopausal woman once again has very little estrogen. Unless she takes supplementation, she will begin to have an atrophic vaginal epithelium that is susceptible to infection, the pH value will rise, and the lactobacilli will begin to disappear. A large survey performed by Dr. Frederick Fleury¹ gives us a fairly good indication of the typical diseases occurring in young women who have complaints of vaginal discharge and vaginitis. Most commonly this group of women had *Gardnerella* vaginitis or nonspecific vaginosis (vaginitis). The next most common infections are with *Candida*, which occur in about 20% to 25% of cases. *Trichomonas* infection occurred in approximately 10% of these women.

Trichomonas vaginalis vaginitis

Symptoms of *T. vaginalis* infection are basically discharge and some itching—but the latter is nowhere near as predominant as the itching associated with *Candida* infections. Examination typically reveals a foamy, bubbly, profuse vaginal discharge that predominates over the pruritus. When the discharge is wiped away, the so-called strawberry cervix—the cervix with a multitude of petechiae—can be seen in 25% to 30% of patients.

Microscopy of a saline suspension from the discharge demonstrates motile trichomonads. Occasionally, isolation methods (Trichicult's or Diamond's) are necessary to identify the presence of *T. vaginalis*.

In the majority of cases the diagnosis is made on the basis of a wet-mount slide in the office. A trichomonad is about halfway in size between a polymorphonuclear leukocyte and a squamous epithelial cell. In differentiating this type of infection from nonspecific vaginitis, it is also important to recognize the multitude of white cells present. In *Trichomonas*, the field is loaded with white cells, and that is a very rare occurrence in patients with nonspecific vaginitis.

Candida albicans vaginitis

Patients with *C. albicans* vaginitis will predominantly complain of vaginal or vulvar itching. They will also have a discharge with a characteristic clumpy, white, cottage-cheese appearance. In addition, there is a reaction to the agent or to products of the agent, producing an erythematous reaction in the vaginal mucosa or on the skin of the perineum. The diagnosis in the majority of cases is made on the basis of a 10% potassium hydroxide smear with identification of pseudohyphae. Culture media are available (Nickerson's, Sabouraud's, and many others) by which the organism can be identified.

Use of either methylene blue or Gram's stain, rather than plain potassium hydroxide, is a much more sensitive method of identifying *Candida*. The most sensitive method is culturing with Nickerson's culture medium.

We also recognize, though, that with Candida there are certain predisposing factors. I believe it is fair to say that we tend to dwell on these factors in the patient with recurrences, but they probably are responsible for a minority of cases. These factors include pregnancy

and the menstrual cycle. Just before the menses is the key time because of peak estrogen and progesterone production during that part of the menstrual cycle. Therefore some of the effects expected in pregnancy are seen at the end of the menstrual cycle.

Oral contraceptives, especially the high-estrogen ones, produce a high glucose effect in the vagina and tend to mimic pregnancy as well. Broad-spectrum antibiotics, which eradicate much of the normal flora of the vagina, and steroids predispose to Candida infections. Diabetic patients, especially those in poor control, are also at risk. Last, although relatively uncommon, Candida can be sexually transmitted.

Nonspecific vaginosis (Gardnerella vaginalis)

Nonspecific vaginosis is caused by a complex synergistic infection involving G. vaginalis and anaerobic bacteria.2 G. vaginalis formerly was known as Haemophilus vaginalis.

The major presenting complaint is of a malodorous vaginal discharge with or without irritative symptoms.

Characteristically, examination reveals a homogeneous vaginal discharge that is adherent to the lateral vaginal walls. The pH value is >4.5. The addition of 10% potassium hydroxide releases a foul, "rotten fish" odor, and a smear from the discharge contains "clue" cells and few leukocytes. Culturing by means of selective media is not necessary as a routine component of the clinical workup.

For a sophisticated approach, one can use gas-liquid chromatography to measure the organic acid metabolites of the anaerobes present in this disease entity.

Nonspecific vaginitis is actually an interesting disease entity in that it is associated with a typical synergistic infection involving both G. vaginalis and anaerobic bacteria. A schematic concept was developed by Dr. Chen³ and associates, in Seattle, Washington, concerning what happens in the vagina to predispose patients to the development of nonspecific vaginosis.

The normal vagina will have abundant lactobacilli as the predominant organism. They control the growth of anaerobes and other bacteria in the vagina by the production of hydrogen peroxide. Not all strains of lactobacilli produce hydrogen peroxide, though. If something interferes with the production of the hydrogen peroxide, the mixed anaerobic and aerobic vaginal flora becomes free to proliferate and grow. G. vaginalis, which is part of the normal vaginal flora in 40% to 60% of women, produces amino acids. Enzymes are produced by the anaerobic bacteria, which leave the amino acids and produce amines. These amines begin to increase the vaginal pH, causing epithelial shedding, which produces the discharge. Thus a vicious cycle starts in which the elevated pH begins to result in cessation of lactobacillus production. The cycle is of progressive disease with vaginal discharge and odor.

The vaginal flora of normal control patients contains Gardnerella in many instances (but it tends to be there in low quantities); lactobacilli predominate, and anaerobes are not prevalent. All women with nonspecific vaginitis have G. vaginalis organisms in extremely high quantities. Anaerobes proliferate and predominate, and lactobacilli begin to disappear.

When a patient responds to therapy, the anaerobes begin to disappear. The high quantities of Gardnerella are greatly reduced, and the lactobacilli begin to return.

In the formal diagnosis of nonspecific vaginitis, the following criteria have been suggested: the presence of a homogeneous vaginal discharge, a pH value above 4.5, the presence of clue cells, and a positive so-called "sniff" test, which results from the presence of potassium hydroxide, which releases a rotten-fish odor.

In the typical homogeneous discharge of nonspecific vaginitis, a "clue" cell is a specific diagnostic indicator. Detection of the clue cell requires attention to certain characteristic features of the cell, First, a clue cell is a squamous epithelial cell whose border contains the adherent Gardnerella organism. Clue cells tend to occur in clumps, with the bacteria located between the squamous epithelial cells. A clue cell is not a squamous epithelial cell whose cytoplasm is covered by bacteria.

Second, it is important to note that there are very few, if any, white blood cells, which is in contradistinction to Trichomonas infection, in which white cells are commonly present.

Finally, it is important, in detecting the clue cell, to note that a normal squamous epithelial cell has a very sharp border, whereas the clue cell loses that sharp border because the organism adheres to the epithelial cell. The typical clue cell has the Gardnerella organism adhering to the border, so that the sharp, distinct border is gone.

The clue cell is important because it signifies not only that Gardnerella is there but that concentrations are very high-more than 107 bacteria per milliliter. Thus the clue cell is a much more sensitive measure of actual clinical infection than are positive cultures for Gardnerella, since 40% to 60% of women have the organism, but not all of these women have vaginosis or vaginitis.

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Epidemiology and pathogenesis of recurrent vulvovaginal candidiasis

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Millions of women worldwide continue to suffer from vulvovaginal candidiasis, which is second only to anaerobic bacterial vaginosis in the United States. Evidence is presented of an increasing incidence of vulvoyaginal candidiasis, the cause of which is unclear, but this increase is probably the result of multiple factors including widespread abuse of antibiotics, possibly oral contraceptives, and most important inadequate vaginal therapy. Some women never experience vulvovaginal candidiasis, others have infrequent episodes, and a third subpopulation have recurrent episodes resulting in considerable morbidity and suffering. Two fundamental questions face investigators: the mechanism whereby asymptomatic colonization converts to symptomatic disease and the elusive explanation for frequent recurrences of vulvovaginal candidiasis. Although several factors have been identified as predisposing to recurrent vulvovaginal candidiasis (pregnancy, oral contraceptives, exogenous hormones, antibiotics, diabetes mellitus, etc.), the majority of women with recurrent vulvevaginal candidiasis do not have recognizable predisposing factors. What has emerged over the last few years is the awareness that different pathogenic mechanisms may be operative in individual patients responsible for a spectrum of clinical manifestations. Understanding the pathogenic mechanisms is essential if we are to progress in treatment. In addition to the study of newer antimycotic agents, new strategies of the rapy are required and must be individualized for patients with recurrent vulvovaginal candidiasis. (Am J OBSTET GYNECOL 1985;152:924.)

Key words: Vulvovaginal candidiasis, recurrent, epidemiology, pathogenesis

Vulvovaginal candidiasis (VVC) is a universal problem affecting millions of women. Second only to anaerobic bacterial vaginosis in the United States, VVC is a widespread cause of vaginal symptoms responsible for untold numbers of prescriptions for antimycotic agents.¹⁻⁴ Although VVC rarely results in hospitalization, it causes not inconsiderable suffering to women and frequently creates severe strains on normal marital relationships.⁵

Approximately three quarters of all adult women suffer at least one attack of *Candida vaginitis*.⁶ It is abundantly clear to physicians that several subpopulations of women may be identified. Some women go through life and never experience a single episode. A second group consists of women with infrequent occasional episodes of varying severity, which rapidly respond to a variety of topical forms of antifungal therapy. A third subpopulation of unknown magnitude is made up of unfortunate women with recurrent, often chronic VVC. Hurley⁷ estimates that 45% of patients have more than one episode of infection.

It is this third group of women, those patients suffering from recurrent vaginal *Candida* infections, that is the subject of this paper. Physicians have been unable to achieve satisfactory therapeutic progress in the management of recurrent infection because of our fundamental failure to advance in our understanding of the pathogenesis of recurrent VVC.

Epidemiology

Point-prevalence studies have established the presence of asymptomatic vaginal colonization with *Candida* in approximately 10% to 55% of healthy adult women. Most investigators, however, estimate the incidence at closer to 15% to 20%. The limitation of point-prevalence studies is the inability to predict the natural history of asymptomatic vaginal yeast carriage. In limited investigations only, asymptomatic carriage persisted for at least 4 weeks and conceivably, in fact probably, continues for much longer. Thus the cumulative lifetime number of women who become colonized for variable periods of time is unknown.

Several host factors have been associated with a higher incidence of vaginal yeast colonization (Table I). Accordingly, asymptomatic colonization has been found in 30% to 40% of healthy pregnant women.³ Similarly, higher carriage rates are described in women receiving oral contraceptives,¹² frequenting venereal disease clinics,¹² and following antibiotic administration.^{13, 14}

When patients are screened or evaluated for the presence of vaginal *Candida*, the interpretation or meaning of a single negative vaginal swab specimen on culture

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should be made apparent to the clinician. In vitro studies by Odds¹⁰ have shown that one needs at least 10³ yeast per milliliter in order to obtain a single colony on agar isolation. Accordingly, the sensitivity of a single negative vaginal swab specimen is such that clinicians might be lulled into a sense of false security by the finding of a negative culture after completion of therapy. When repeat cultures are reported positive some 30 days later, as is the practice in therapeutic trials, this is attributed to vaginal reinfection rather than vaginal relapse.

The typing system of Odds and Abbott,15 which permits "finger printing" and, hence, identification of specific strains of Candida albicans, has provided valuable information in the epidemiology of asymptomatic and symptomatic VVC. Although more than 200 vaginal strains have been identified, the majority of patients are colonized by a more limited number of strains. Studies in the United Kingdom and the United States by Odds et al. 16 indicated that vaginal strains of C. albicans could be grouped into fewer than 20 clusters with common bacterial properties. This observation might suggest that certain "vagina-tropic" or "vaginopathic" strains of Candida might be selected for both vaginal colonization and vaginitis by virtue of certain virulence characteristics. However, the same frequency of strain prevalence was found when strains from different anatomic sites were analyzed. 10 Moreover, no difference in strain types was observed when vaginal isolates from asymptomatic and symptomatic women were compared.10 Only minor differences in strain frequency were found when United States and United Kingdom isolates were compared.16

The pathogen

Well over 80% of yeast isolated from the genital tract of asymptomatic and symptomatic women throughout the world are *C. albicans* strains. ^{12, 17, 18} The remainder are due to other Candida species, primarily *Torulopsis* (Candida) glabrata, which accounts for 3% to 16% of isolates. Although considered less virulent than *C. albicans*, *Torulopsis glabrata* has been associated with frank vaginitis. Experimental studies in humans and animals define the incubation period as 24 to 96 hours following vaginal inoculation of Candida. Infection may be induced with an inoculum as small as 10² microorganisms.

Usually in an experimental study, the vagina is inoculated with an inoculum of 10⁷ microorganisms. With an inoculum of 100 microorganisms, a significant vaginitis can be caused. This has to be borne in mind when one considers the sites of spread or the source of vaginal infections, whether by sexual transmission or from another extravaginal source.

Table I. "State of the art" summary of pathogenesis (1970s)

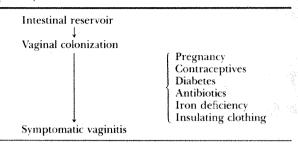


Table II. Pathogenesis of recurrent VVC in pregnancy

- 1. Asymptomatic colonization ↑
- 2. Experimental studies of Bland
- 3. Higher glycogen content (hormone effect)
- 4. Candida hormone receptors
- 5. Enhanced cellular susceptibility
- 6. Symptomatic vaginitis in 60%-90% of pregnant carriers
- 7. Symptomatic vaginitis \(\gamma \) with duration of gestation
- 8. Recurrence more frequent
- 9. Clinical response \$\diamsup\$

Pathogenesis of recurrent vulvovaginal candidiasis

Several factors have long been recognized as predisposing causes of recurrent *Candida* vaginal infection.

Pregnancy (Table II). Vaginal yeast carriage is more frequent in pregnancy and the prevalence continues to increase with the duration of the pregnancy. Is. 49 In a unique study investigating the high susceptibility of pregnant women to vaginal yeast infection, Bland et al. 20 inoculated the vaginas of pregnant and non-pregnant women, inducing infection in more than 80% of the former and only a third of the latter.

The mechanisms by which pregnancy encourages colonization are complex. Most authors attribute the high colonization frequency and high attack rate to increased vaginal epithelial glycogen content under the stimulation of reproductive hormones. However, the problem is more complex than the availability of a carbon source which certainly enhances yeast growth, multiplication, adherence, and germination. The recent identification of receptors in the cytosol of *Candida* for estrogens and progesterone together with the observed stimulatory effect of these hormones directly promoting *Candida* and germination points to the direct virulence-enhancing effects of female reproductive hormones. Effects

Not only are the hormones influencing the environment, the pH, and the glycogen content, they are directly affecting both the yeast and probably also the receptor sites and adherence of the individual vaginal epithelial cells.

The finding of hormone receptors in yeast may well explain the exquisite sensitivity and dependence of the rat model of experimental vaginal candidiasis on estrogen injection. Similarly, studies in our laboratory reveal that *C. albicans* adhere in significantly greater numbers in vitro to exfoliated vaginal epithelial cells obtained from estrogenized as opposed to oophorectomized, estrogen-deprived rats.

During pregnancy, the percentage of vaginal carriers who manifest symptoms of overt vaginitis is also considerably higher among pregnant than nonpregnant women. Symptomatic vaginitis was observed in 60% to 90% of pregnant vaginal carriers. Thus the incidence of symptomatic VVC is high in pregnancy and increases during the course of gestation.

Symptomatic VVC developed in about 10% of pregnant women during the first trimester. 4. 5. 18 Furthermore, recurrent attacks are common with almost half of the symptomatic women suffering a second episode during the same pregnancy. 5. 17 Not surprising, therefore, is the observation that cure rates are lower during pregnancy.

Oral contraceptives. Although there are several publications reporting the increased frequency of both asymptomatic vaginal carriage of yeast and VVC, the precise role of oral contraceptives in predisposing to symptomatic vaginal infection remains controversial. 3, 12, 24 Most epidemiologic studies have coumented at least an increased colonization rate of 20% to 45%. 3, 12 Once more, the increased carriage is thought to result from the effects of female hormones or epithelial cell adherence or receptivity or from the glycogen and substrates available to the microorgamisms as well as the direct effect of oral contraceptives on yeast virulence.

Recent studies, however, have not unequivocally demonstrated an increased rate of symptomatic Candida vaginitis, particularly in users of low-estrogen preparations.¹²

Antibiotics. Virtually no antibiotic is immune from this frequent complication although broad-spectrum agents such as tetracycline, ampicillin, and cephalssporins appear to be the commonest offenders.

Antimicrobials are thought to act by reducing the normal protective resident bacterial population, particularly lactobacilli species, and hence reducing the natural colonization resistance. In two clinical studies the prevalence of vaginal carriage increased from about 10% to approximately 30% after 2 to 3 weeks of treatment with tetracyclines even at low dosages. ^{13, 14} The increased prevalence of vaginal yeast carriage is usually accompanied by a simultaneous increase in *Camidu*positive cultures obtained from the gastrointestinal tract. The postantibiotic increase in vaginal *Candian* re-

flects either increased spread in the number of previously unrecognized vaginal *Candida* which multiply in the presence of a less inhibitory environment. Studies of the gastric flora of rats reveal that lactobacilli and *C. albicans* exist in harmony, each species colonizing anatomically distinct areas based upon specific histologic differences.²⁵ Lactobacilli occupy the keratinized, stratified squamous epithelia and *Candida*, colonize, and are attached to secretory mucosal cells. After oral administration of tetracyclines, the lactobacilli are eliminated and the *Candida* organisms multiply to colonize the entire mucosal surface. After cessation of treatment and administration of lactobacilli, the original microflora of the stomach is reestablished and the two populations continue their symbiotic relationship.²⁵

Not only are antibiotics responsible for the increased frequency with which women are colonized with Candida today, but antibiotics are probably the most commonly identified precipitating factors of vaginitis. Many patients note the onset of symptoms of VVC during the course of therapy with antimicrobials. The rapidity with which symptoms appear after oral administration of antibiotics suggests not only that vaginal colonization resistance mechanisms are depleted but possibly that antibiotics actually stimulate yeast proliferation. Although only a few studies have been done to investigate this issue, it appears reasonable to recognize the dangers of administering antibiotics to high-risk patients and to consider concomitant antifungal therapy.

Diabetes mellitus. Uncontrolled diabetes mellitus with accompanying glycosuria and increased glucose concentrations in vaginal secretions may precipitate frank symptomatic vaginitis. Clinicians invariably consider subclinical or unrecognized diabetes in patients with recurrent VVC and subject these women to 3- and 5-hour glucose tolerance tests. The yield of these tests is extremely low and does not justify the perpetuation of this practice except in postmenopausal women. It is extremely unlikely in an otherwise healthy woman that the only manifestation of hyperglycemia will be recurrent VVC.

Some women with recurrent VVC attribute recurrences of VVC to dietary indiscretions, for instance, sweet and candy binges, possibly inducing transient glycosuria.

Iron deficiency. The studies of Davidson et al.²⁶ appear to have clarified the issue as to whether iron deficiency predisposes to VVC, in that no positive evidence incriminating iron deficiency was found.

Clothing and personal habits. Many women have identified tight, insulating clothing, particularly nylon underwear, and pantyhose as factors that frequently precipitate symptomatic bouts of VVC. Poor ventilation and increased temperature moisture of the perineum

encourage yeast proliferation. Similarly, although a wet bathing suit is thought to bring on symptoms, it is probably the chlorine in swimming pools that irritates the vaginal mucosa and increases the likelihood of symptoms developing. Many women feel that deodorant sprays, perfumed toilet paper, and commercial douches similarly act to exacerbate symptoms. The role of vaginal tampons in causing minor friction of the mucosa and precipitating attacks has not been clarified. All the above factors may act to sensitize the mucosa to the pathogenic mechanisms of resident yeast in the vagina and induce symptoms.

Miscellaneous factors. The role of hypothyroidism, stress, and frequency and technique of sexual intercourse remains undetermined. Many sufferers of recurrent infections describe a correlation between infrequent but often intensive sexual relations and the precipitation of a symptomatic episode, suggesting that local minor trauma resulting from friction may create conditions suitable for commensal yeast to invade superficial tissue. On questioning of patients, about 15% will give a positive history connecting sexual intercourse to symptomatic Candida infections. In a small minority of patients with a definite cause-effect history, postcoital prophylaxis with a single dose of clotrimazole may be worth trying. Studies need to be done looking at this particular entity. It has been shown to be effective in women who have recurrent urinary infections where there is a clear connection between intercourse and cystitis. In a study commissioned by the C. B. Fleet, Co., Inc., to determine the microflora residing in douche equipment, C. albicans was isolated from 21% of the nozzles and 2.5% of bags or bulbs surveyed.

Ironically, women with recurrent VVC almost invariably lack these risk factors and in spite of avoidance of all known risk factors continue to suffer from recurrent vaginal Candida infections. These unfortunate women, aware of publicized precipitating factors, avoid oral contraceptives and other exogenous hormones, avoid pregnancy, use appropriate clothing, have normal glucose tolerance tests, and, finally, avoid antibiotics at all costs and still attacks recur. Thus the critical question regarding the mechanism of recurrent VVC remains unanswered.

Current theories regarding source of recurrent VVC

Intestinal reservoir. The commonest quoted theory is that of a persistent intestinal reservoir of Candida that results in recolonization of the perianal area and recurrent vaginal reinfection.27,28 In one study conducted by Miles et al.28 virtually 100% of women with recurrent VVC had simultaneous positive rectal and vaginal cultures. Other authors,3 however, have failed to confirm

this uniform concordance. Similarly, in our clinic only 69% of women with recurrent VVC presenting with an acute exacerbation of VVC had simultaneous positive rectal cultures. After long-term treatment with oral ketoconazole, only 40% of patients with posttreatment recurrence of VVC had positive rectal cultures. Likewise Milne and Warnock29 in a controlled prospective study were unable to correlate symptomatic recurrences with positive rectal cultures. Furthermore, many chronic fecal carriers of yeast appear to be resistant to vaginal colonization with Candida.27 Numerous studies with long-term oral nystatin therapy aimed at preventing vaginal reinfection have failed to reduce recurrence rates.29,30

In spite of the data arguing against vaginal reinfection from the perianal region as a source of vaginal recolonization, it should be pointed out that, with the Candida strain typing method of Odds and Abbott,15 the majority of vaginal Candida strains are identical to those isolated from the patient's oral cavity and rectum. Similarly, with the same method of strain typing, we have observed vaginal and gut strain concordance in more than two thirds of patients with simultaneously positive cultures. Thus in a large number of those patients identical strains are found and additional prospective studies are required to clarify this controversial issue. It would appear that the gastrointestinal reservoir is a potential source of reinfection, but its importance may well have been exaggerated. This is not a mute issue since therapeutic strategies for the future which include oral therapy with nystatin or ketoconazole require critical evaluation.

Sexual transmission. The role of sexual transmission as a means of vaginal inoculation and colonization has undergone considerable discussion in the last few years. Once more, studies have identified asymptomatic penile yeast carriage, usually in the coronal sulcus, in approximately 5% to 25% of male partners of women with symptomatic VVC. 10, 81, 32 Yeast penile colonization is fourfold more prevalent among male sexual partners of infected women than among other men from the same population.33 About 80% of female contacts of infected men have positive yeast cultures versus only 32% of women with uninfected partners.33 Typing the strains from both partners reveals that in the majority of cases both members harbor the identical strain.16, 34, 35

The above data are, however, only circumstantial evidence incriminating the male genitalia as a source of vaginal colonization and reinfection since the penis may be colonized passively during intercourse and low titers cultured may be incapable of infecting the vagina. Thus unequivocal proof that the colonized penis may transmit infection is still lacking. Even if sexual transmission



Fig. 1. Candida balanoposthitis.

is a source of vaginal colonization and accepting the maximum penile colonization rate of 25%, this infers that in no more than 25% of women with recurrent VVC could vaginal reinfection be attributed to sexual transmission. Given these numbers, together with the fact that many women who develop both infrequent and recurrent VVC are sexually inactive, the overall contribution of sexual transmission to vaginal yeast colonization is probably relatively limited. Although some clinicians emphasize the necessity for routine topical penile treatment of all partners of symptomatic women with VVC, this approach requires confirmation in prospective controlled comparative studies. 36 Although orogenital and anogenital contact have been suggested as risk factors, further epidemiologic investigations including typing studies are required to confirm a causal relationship.

Of 100 women investigated at our clinic there have been three male partners who developed and presented with frank Candida balanoposthitis (Fig. 1). This is an acute inflammatory reaction, which resembles the vulvitis seen in women. More commonly, 15% or 20% of male partners develop the phenomenon of an acute hypersensitivity reaction. They develop a severe itch and redness shortly after intercourse, which disappears by the next morning. Usually, the male partner is culture negative indicating an entirely different mechanism of a contact dermatitis. Whether or not it is due to a form of acute hypersensitivity, it is not balanoposthitis. It clearly indicates that more than one mechanism is responsible for the development of symptoms in both sexes.

Vaginal relapse. Another important consideration is

that recurrence of symptomatic vaginal infection may result from failure of conventional therapy to completely eradicate Candida from the vaginal lumen and possibly the superficial vaginal mucosal tissue. Transmission electron microscopic studies of exfoliative human vaginal and cervical epithelial cells have shown that both the Candida blastospore and particularly the germinated microorganisms are capable of invading intact epithelial cells in the superficial vaginal mucosal tissue. Transmission electron microscopic studies of exfoliative human vaginal and cervical epithelial cells have shown that both the Candida blastospore and particularly the germinated microorganism are capable of invading intact epithelial cells in the superficial layers of the mucosa and appear capable of penetrating to the depth of several layers³⁷ (Fig. 2). The implications of this intracellular phase are obvious since it suggests that organisms may sojourn within the mucosa protected from antifungal agents, only to reemerge into the vaginal lumen some weeks or months later, when the epithelial cells are normally shed under the maturation process that occurs each month. This hypothesis of a persistent intracellular phase of Candida in vivo requires verification but may well explain the occurrence of positive vaginal cultures after treatment and several interim negative vaginal cultures in sexually inactive, rectal culture-negative women.

Odds,3 in reviewing the literature, concluded that approximately 20% to 25% of women responding clinically to standard antifungal topical measures with negative vaginal cultures at completion of therapy were found to have positive vaginal cultures within 30 days. This is usually interpreted as reinfection, but it may be true relapse. Utilizing an animal model of experimental Candida vaginitis in rats, we have similarly observed the appearance of positive vaginal cultures 30 and 60 days following initial, supposedly successful treatment with oral ketoconazole. The rats were sexually inactive, had negative rectal cultures, and had identical strains of C. albicans isolated on each occasion. Longitudinal studies in our clinic with patients receiving long-term oral ketoconazole revealed that more than half the patients with posttreatment recurrence had identical strains identified with successive vaginal cultures. Accordingly, the cumulative data strongly suggest that, in a substantial number of women with repeated episodes of VVC, recurrence of colonization and symptomatic infection is due to failure of therapy to completely eradicate all yeast from the vagina. Once more, this premise has important therapeutic implications especially given the lack of sensitivity of a single vaginal swab to detect small numbers of Candida organisms.10 Furthermore, therapeutic regimens should be evaluated not only in terms of successful relief of acute symptoms but by longterm follow-up studies of vaginal Candida recoloni-

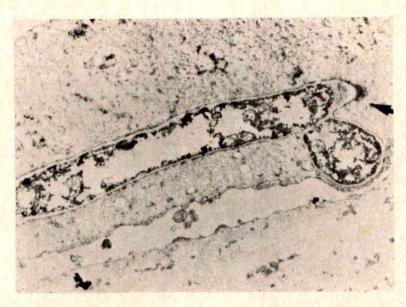


Fig. 2. Invasion of intact epithelial cells by the Candida blastospore. (Reproduced with permission from García-Tomayo J, Castillo G, Martínez AJ. Human genital candidiasis: histochemistry, scanning and transmission electron microscopy. Acta Cytol [Baltimore] [1982;26:7].)

zation rates. Thus it is particularly disturbing that investigators38 have reported even higher vaginal yeast recovery rates following short courses of therapy.

Irrespective of the source of vaginal recolonization which may, therefore, differ in different women, why do some women have infrequent episodes and others continue almost on a monthly basis to be plagued by recurrent symptomatic episodes in the absence of recognized predisposing factors? The answer to this important question may reside with the host's normal vaginal defense mechanisms directed by both a preventing vaginal colonization and exacerbation of symptomatic disease.

Host defense mechanisms directed at C.

Relatively little is known about the natural vaginal mechanisms that resist Candida colonization and symptomatic infection.

Genital tract antibodies. Clinically, VVC is not associated with any form of immunoglobulin deficiency. Both serum and local cervical antibodies to C. albicans have been identified in both symptomatic and asymptomatic vaginal candidiasis. 39-43 Titer changes both locally and systemically are unreliable in diagnosis. 40 The dominant anticandidal immunoglobulin is IgA, particularly in cervical secretions (SIgA). In the gastrointestinal tract IgA antibodies are thought to prevent mucosal colonization by agglutination of microorganisms and reduction of adherence to intestinal cells. Although cervical anti-Candida IgA may have the same effect, any protective efficacy is unproven. Women may have either chronic asymptomatic colonization or recurrent symptomatic vaginitis with the same strain of Candida in the presence of copious cervical antibodies. 42 The role of candidal antigenic heterogeneity or the production of immunoglobulin-cleaving enzymes by the fungi (proteolytic) in negating the protection of antibody is unknown. Vaginal titers are lower in patients from whom yeast are cultured. The relevance of this observation and the protective status of anticandidal antibodies are unknown.

Cell-mediated immunity. Although immunocompromised patients receiving steroids and other immunosuppressive agents are at higher risk of developing symptomatic VVC, the role of cell-mediated immunity in preventing vaginal colonization is unclear, particularly because of the superficial nature of the mucosal yeast infection. Almost all healthy adults, noncolonized, colonized, and with symptomatic VVC, have a normal cutaneous delayed hypersensitivity reaction to the introduction of candidal antigen. Similarly, in the vast majority of women, including those with recurrent VVC, in vitro lymphocyte studies show normal stimulation indices following exposure to mitogens. 43, 44 When Candida antigen is used to stimulate lymphocyte proliferation, conflicting results have been obtained. Some authors43, 44 have detected, in women with recurrent VVC, a specific diminished in vitro lymphocyte stimulation in response to Candida antigen only. In other words, they may actually have defective in vitro response to T-cell antigens. This does not mean a preexisting T-cell defect, but rather the Candida in itself may suppress populations of T lymphocytes and create a form of tolerance. In order to get rid of the tolerance, the Candida antigen has to be eliminated or the immune

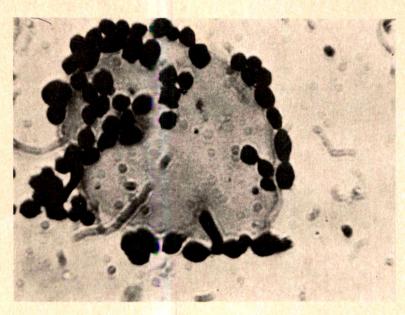


Fig. 3. Blastospores of Candida albicans adhering to a vaginal epithelial cell.

response improved. Additional studies are required since a highly specific immune defect which results in susceptibility to mucosal *Candida* infection in one site only and confined to one species of microorganism without manifestations elsewhere in the body is unlikely.

Phagocytic cells. Although phagocytes constitute an important defense mechanism combating systemic and deep-seated fungal invasion, there is no evidence to implicate phagocytes as a superficial vaginal mucosal defense mechanism. 45 Leukocytes are typically absent from vaginal secretions in frank candidal vaginitis, which may be a function of the superficial nature of the mucosal infection or the lack of chemoattractants generated in the inflammatory process.

Vaginal flora. The normal bacteria that populate the vagina probably constitute the most important natural defense mechanism against Candida colonization. Experimental studies of germ-free mice revealed the ease with which the animals undergo yeast gastrointestinal colonization, in contrast to nongnotobiotic animals.46 The protective role of vaginal bacterial flora has been emphasized by the rapid acquisition of positive vaginal cultures as well as the onset of symptoms following local and systemic administration of antibiotics. Either the reduction or the alteration of the bacterial population permits newly introduced yeast to establish themselves or when small numbers of yeast are already present they then multiply freely and more extensively colonize the vagina. Lactobacilli have been singled out as having the dominant role in preventing both the establishment of vaginal candidiasis and the subsequent development of symptomatic VVC.3 It is of interest, therefore, that decreased numbers of lactobacillus species have been found in patients with symptomatic vaginitis. 47 C. albicans has the potential to elaborate substances that are active against bacteria; thus the meaning of reduced titers of lactobacilli as a cause or result of Candida infection is unclear.48 The most commonly quoted explanation of bacterial antagonism of yeast is competition for nutrients and hence the fungal population is kept in check by the alleged limited availability of nutrients. This is probably an oversimplification and more likely other factors, such as bacterial production of Candida growth-inhibitory factors observed in in vitro studies with lactobacilli and other gram-negative rods, play an important role.3 In my laboratory candidal attachment to exfoliated vaginal epithelial cells was reduced when the cells were preincubated in the presence of lactobacilli.49

Candida virulence factors in the pathogenesis of recurrent VVC

So far, all discussion has centered on the role of those factors, both endogenous and exogenous, in the pathogenesis of recurrent infection. The role of the pathogenic microorganism is largely undetermined. There is no evidence that chronic or recurrent VVC is due to the development of antimicrobial resistance following repeated courses of therapy. It may well be asked whether *Candida* vaginal isolates associated with asymptomatic colonization are less virulent than those cultured from patients with symptomatic infection and, conversely, are organisms responsible for recurrent infection more virulent?

From an epidemiologic point of view, Odds¹⁰ has found an identical distribution frequency of vaginal clinical isolates for *Candida*, based upon strain typing

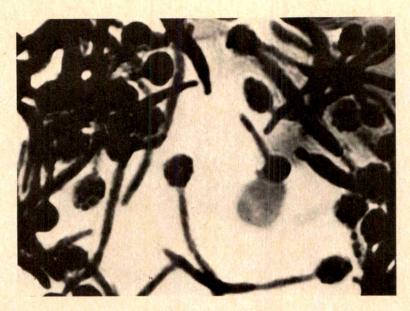


Fig. 4. Enhanced adherence of Candida albicans in the presence of germ tube hyphae formation.

in patients with both symptomatic and asymptomatic states. Vaginal strains of C. albicans had a similar distribution frequency when compared to isolates obtained from other clinical sites.10

Virulence factors of Candida remain for the most part unestablished. The first virulence factor we have investigated is that of microbial adherence to mucosal epithelial cells49 (Fig. 3). After introduction into the vagina, usually as blastospores, the microorganisms must have the capacity to adhere to the mucosa, a function that is both enhanced and facilitated by in vivo germination.49, 50 Thereafter, the organisms must be able to multiply and sustain themselves nutritionally in the face of competition from the local bacterial flora. Finally, to survive even as commensals, Candida must withstand any attempt on the part of normal vaginal resistance mechanisms to discourage yeast presence. As mentioned previously, based upon typing studies there is no satisfactory evidence to suggest that C. albicans strains differ in their capacity to colonize the vagina.10 In vitro studies found no significant difference in adherence to exfoliated vaginal epithelial cells of C. albicans isolates obtained from different anatomic sites. 49 In contrast, similar experiments have shown that not all Candida species adhere as well as C. albicans. Thus C. tropicalis, C. parapsilosis, and C. kruzei adhered in significantly lower numbers of vaginal cells; this may well explain the lack of vaginal tropism of these organisms and the infrequency with which they are found on vaginal culture.50

Since adherence to vaginal epithelial cells is increased when Candida blastospores germinate, in vivo germination may also be important in vaginal colonization, even though germinated yeast are infrequently seen in vaginal smears obtained from asymptomatic culturepositive women¹² (Fig. 4). Using a mutant strain of C. albicans incapable of in vivo germination at 37° C, Sobel et al.51 demonstrated that rat vaginal colonization was significantly reduced with the mutant strain and spontaneous vaginal clearance of this organism was observed. Both characteristics, i.e., fungal capacity to adhere to target cells and to germinate, are important veast virulence factors; however, there is no evidence that selection of pathogenic strains in vivo is dependent upon these criteria. Recently, Crandell et al.52 in experimental studies detected a non-protease-producing C. albicans mutant with reduced ability to adhere to endothelial cells.52

There is surprisingly little knowledge available as to the mechanisms whereby saprophytic yeast are stimulated or activated to increase in number and reach a more virulent phase capable of inducing inflammation.

Symptoms of VVC are not strictly related to the yeast load. Thus Oriel et al.12 recovered 103 to 104 yeast per milliliter of vaginal fluid in symptomatic and asymptomatic states. Certainly many clinicians have noted that some severely symptomatic women may have few yeast apparent in the vaginal secretions whereas not infrequently one may see the converse, that is, large numbers of yeast (even germinated) in wet mounts obtained from asymptomatic women. Nevertheless, in spite of these exceptions, the majority of women with symptomatic disease have large numbers of yeast present in secretions as well as evidence of in vivo germination. The discrepancy in these observations could result from patient subjective tolerance or sensitivity to the toxic mechanisms of pathogenic yeast or because yeast must act in concert with other factors such as bacteria

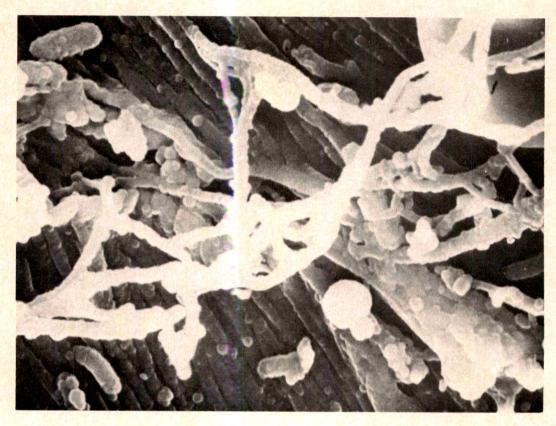


Fig. 5. Scanning electron micrograph demonstrating hyphae formation and invasion of vaginal mucosa.

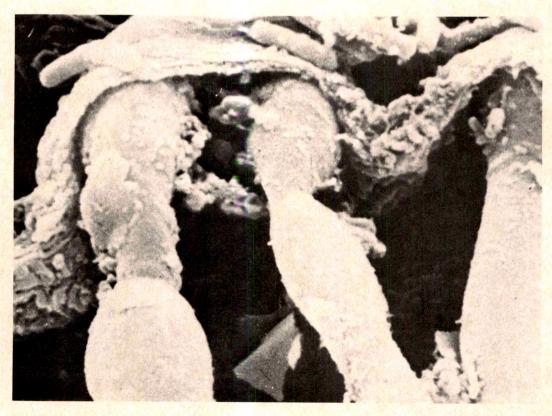


Fig. 6. Scanning electron micrograph of the surface of the vaginal mucosa infected with *Candida albicans*. (Courtesy of Marcel Boyers, Ph.D., Janssen Pharmaceutica, Beerse, Belgium.)

in causing inflammation, i.e., polymicrobial induced inflammation.53

There is a strong association between active clinical disease and the presence of germinated or filamentous Candida³ (Fig. 5). Germinated yeast adhere more avidly to vaginal epithelial cells, enhance colonization, and represent the dominant histologically proven fungal form in superficial but invasive Candida vaginitis as well as in disseminated systemic candidiasis. Several studies using scanning and transmission electron microscopy have demonstrated the invasive capacity of germinated yeast in humans and animals3,37 (Fig. 6). Studies in our laboratory revealed that the nongerminating mutant of a wild strain of C. albicans was noninvasive and caused mild vaginitis only.51 Thus germination appears to be a critical virulence factor in the pathogenesis of VVC.

Factors favoring germination can be expected to be associated with the precipitation of symptomatic vaginitis. We have reviewed many clinical isolates of Candida obtained from patients with acute symptomatic vaginitis. All the latter were capable of in vitro germination as were isolates from asymptomatic patients and isolates from numerous anatomic sites. Thus, while germination appears to be an important yeast virulence factor in the initiation of mucosal invasion, as a property in Candida species it is almost invariably present in all clinical isolates. Appreciation of the significance of germination has therapeutic implications in that imidazoles capable of inhibiting germination at low concentrations might be of value in preventing recurrent VVC. Preliminary studies by Sobel and Muller⁵⁴ using the rat model of experimental VVC suggested that low-dosage ketoconazole, providing subtherapeutic vaginal levels of the imidazole, was effective in preventing vaginitis possibly by the effect of germination inhibition (Fig. 7). In spite of our awareness of the significance of germination in invading mucosa, transmission electron microscopic studies have shown that blastospores of Candida are also capable of invading epithelial cells.37

The mechanism by which Candida produce disease is unknown. The extensive areas of pruritus and inflammation often with minimal invasion of cells suggest an extracellular toxin or enzyme. In spite of the evidence of cell invasion described above, it should still be emphasized that Candida vaginitis is primarily a mucositis or superficial infection, to the extent that, in the past, clinicians considered it an infection of vaginal secretions. In vitro C. albicans elaborates an acid protease optimally active at pH 4.0 to 4.5, i.e., the normal vaginal pH and the pH most frequently associated with VVC. Additional studies are needed to further clarify how yeast induce inflammation. Some men develop penile irritation within a few hours of sexual contact with infected asymptomatic women. This suggests some form

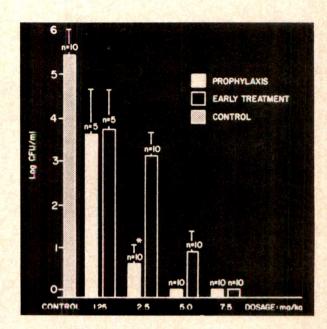


Fig. 7. Comparison of efficacy of ketoconazole prophylaxis and early treatment regimen in early candidal vaginitis. (Reproduced with permission from Sobel JD, Muller G. Ketoconazole prophylaxis in experimental vaginal candidiasis. Antimicrob Agents Chemother [1984;25:281-2].)

of irritative or hypersensitivity phenomenon which could also play a role in women.

Based on the above observation, it is conceivable that different pathogenic mechanisms may be responsible for inducing different symptom complexes in individual patients. From a clinical point of view, some women present with a dominant Candida vulvitis with no apparent vaginitis and the pathogenesis is in all likelihood different from that in those patients presenting with a more exudative vaginitis characterized by a cheesy white discharge. Efforts should be made to reclassify recurrent and chronic vulvovaginal candidiasis as to the basis of anatomic site as well as the dominant pathogenetic mechanism.

In conclusion, while the goal of clinicians is to establish successful therapeutic regimens capable of controlling if not curing recurrent VVC, most of our efforts have been directed at studying new antimycotic agents. Not surprisingly, therefore, little progress has been made in controlling these problem patients. Effort must be made to further our understanding of basic mechanisms responsible for recurrent infection. What has emerged is the awareness that although predisposing factors and precipitating mechanisms are recognized, such known factors are more often than not absent in patients with recurrent VVC. The source and mechanism of vaginal recolonization in women with recurrent VVC continue to be controversial and largely unexplained. Sexual transmission probably plays only a small role and the intestinal reservoir concept appears to have been exaggerated. Vaginal persistence and the

concept of vaginal relapse are more likely explanations, although different mechanisms may be operative in different patients. Similarly, our knowledge of mormal vaginal defense and immune mechanisms remains incomplete and additional studies are needed. There is only scant information available concerning the pathogenesis of symptoms experienced and current evidence suggests more than one mechanism is involved.

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Classification and pathogenesis of vulvovaginal candidiasis

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Although candidiasis of the female genital tract is one of the most common of the vaginitides, it is a poorly understood disease entity. Vulvovaginal candidiasis is a monoetiologic disease, but the pathways by which pathogenic expression is attained are sufficiently divergent to constitute a classification schema that influences therapy. For selection of appropriate therapy, the following three broad categories are proposed: (1) primary candidiasis, (2) antibiotic-induced candidiasis, and (3) systemically induced candidiasis. (AM J OBSTET GYNECOL 1985;152:935.)

Key words: Vulvovaginal candidiasis, classification, treatment

Although candidiasis of the female genital tract is one of the most common of the vaginitides, it remains one of the most poorly understood.

Significance of recovery of Candida albicans

The female genital tract is a sophisticated microbiologic environment. The microbiologic presence of an organism does not necessarily equate with disease. Depending on the age group, geographic location, and socioeconomic status, up to 44% of women may harbor one or more species of Candida as a "normal" constituent of the vaginal flora (Table 1). The magnitude of organismal replication is in the order of 102-105 colonyforming units (CFU) per milliliter of vaginal fluids (unpublished observations). In most patients it is only with the application of quantitative microbiology that differences appear between asymptomatic carriers and patients with overt disease. The emergence of disease from a state of prior colonization is accompanied by a quantitative change in the magnitude of organismal replication (greater than 10⁵ CFU/ml of vaginal fluid [unpublished observations]).

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Classification of vulvovaginal candidiasis

The purpose of classification is to impose order on a complex set of facts. Although candidiasis of the female genital tract is a monoetiologic disease, the pathways by which a possibly normal constituent of the genital-anal microbiologic flora attains pathogenic expression are sufficiently divergent to constitute a basis for classification.

For selection of appropriate treatment of a specific candidiasis, the following three broad categories are proposed: (1) primary candidiasis, (2) antibiotic-induced candidiasis, and (3) systematically induced candidiasis.

Monif classification of vulvovaginal candidiasis

- Genesis I. Primary candidal vulvovaginal candidiasis
 - A. Vulvitis*
 - B. Vulvovaginitis with predominantly vulvar involvement
 - C. Vaginitis with relatively minimal vulvar involvement
- Genesis II. Antibiotic-induced vulvovaginal candidiasis

*As an isolated entity, it is more likely in diabetic patients who have cutaneous candidiasis or who have had it in the past.

Table I. Prevalence of Candida albicans in asymptomatic female patients

Source	Type of population (No. of obstetrics age group)	Incidence of colonization
Adler et al. ²	Antenatal, gynecology, and family planning clinics, England 1838 (16-59 vr old)	96/1000
Elegbe and Modupe ³	Undergraduate students, Univer- sity Life, Nigeria (15-22 yr old)	445/1000
Armstrong and Wiesher ⁴	Veneral disease clinic (n = 57), age grouping (not identified)	405/1000
Schnell ⁵	Urban German population (not identified)	113/1000

- A. Resulting from systemic therapy for nonvulvovaginal infection
- B. Resulting from local or systemic therapy for vaginal infection (Ping-Pong vaginitis)

Genesis III. Systemically influenced vulvovaginal candidiasis

- A. Pregnancy, high-dose estrogen, and contraceptives
- B. Steroids
- C. Diabetes mellitus
- D. T-cell dysfunction
 - 1. Congenital
 - 2. Acquired

Vulvovaginal candidiasis (genesis IA to IC: Primary). Augmented moisture and humidity appear to be the principal catalytic factors in primary candidal vulvovaginitis. This disease entity is not totally homogeneous but, rather, is a spectrum of diseases whose "dipoles" are vulvitis at one end and vaginitis at the other. Idiopathic vulvovaginal candidiasis is more a vulvitis than a vaginitis. The principal manifestation of vulvovaginal candidiasis is pruritus, not discharge. The presence of a noncharacteristic discharge should alert one to the probability that the disease entity may not be candidiasis. When discharge indicative of significant concomitant vaginitis is present, it tends to be relatively sparse in quantity and to have a characteristic cottage cheese-like appearance. Vaginitis predominating over vulvitis is more characteristic of antibiotic-induced candidiasis.

The distinction between those cases in which vulvitis is greater than vaginitis and those in which vaginitis is greater than vulvitis is of more than academic importance. Total reliance on intravaginal medication in cases with significant vulvitis is associated with frequent therapeutic failures. If vulvitis is clinically significant, simple eradication of a vaginal reservoir will not afford relatively prompt relief of symptoms nor a one standard deviation probability of a microbiologic cure (unpublished observations). The physicians must concomitantly treat the patient for vulvitis. Treatment is best

achieved through direct application of medication to the region, restriction of undergarments to loose-fitting cotton pants, and use of nystatin powder for the perineum. The use of nystatin, however, may constitute "overkill" in terms of expense.

Anorectal extension of disease is one situation in which the use of oral nystatin may be warranted. Unfortunately, this therapeutic recommendation is not handicapping by data derived from a well-controlled double-blind, randomized study.

Subpatterns of disease. Primary vulvovaginal candidiasis is associated with a defined pH range of 4.2 to 4.7. Analysis of wet-mount preparations reveals two patterns. In one case there are many squamous cells, some pseudohyphae, and rare polymorphic neutrophils. The other pattern is characterized by the concomitant presence of many polymorphic neutrophils, as well as visual evidence for significant mycotic replication. The reason for the divergence is usually related to elaboration of bacteriocins by selected strains of Candida. Even without bacteriocins, pH is the principal regulator of any bacteria that may be present in significant numbers (the latter defined as greater than 10⁵ CFU/ ml of vaginal fluid). Bacteria such as the group B Bhemolytic streptococci and selected members of the Enterobacteriaceae are capable of functioning well in a relatively acid microbiologic environment. The group B streptococci will adhere to squamous epithelial cells and hence appear as so-called "clue cells." The identification of clue cells has fostered a false conceptual association between Gardnerella vaginalis and Candida albicans. The microbiologic environment necessary to sustain one as a vulvovaginal pathogen effectively precludes functional significance for the other agent, although it may be concomitantly isolated. Quantitative studies indicate that under conditions for either organism, the colony counts of the suppressed agent are near the threshold of detection (less than 10^s CFU/gm of vaginal fluid). The inflammatory response elicited is ultimately the mechanism that alters pH and in so doing allows for emergence of anaerobic bacteria.

Vulvovaginal candidiasis (genesis IIA: Antibiotic-

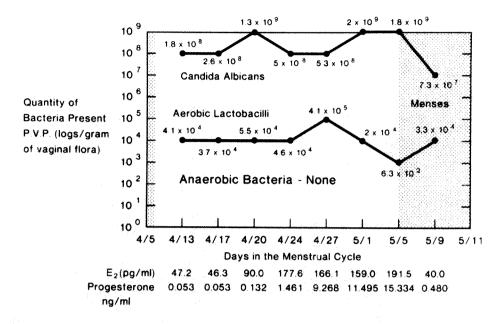


Fig. 1. Quantitative characterization of the aerobic and anaerobic bacterial flora from a patient with persistent chronic vulvovaginitis caused by *C. albicans*.

induced Candida vaginitis. Why do antibiotics induce candidiasis? To be effective in inducing candidiasis, the antibiotic must alter the microbiologic environment through the eradication of the principal anaerobic bacteria that govern pH.7-10 The inhibitory effects of bacteriocins and bacteriocin-like products are pH dependent.9 The eradication of certain bacteria, especially anaerobes, in selected cases frees Candida from its replicative constraints. Antibiotic-induced candidiasis manifests primarily as a vaginitis when the magnitude of replication exceeds 106 CFU/ml of vaginal fluid and the Candida organisms revert to their tissue-invasive dimorphic form. Vaginitis is more characteristic of this group than is vulvitis. Just as Candida is susceptible to bacteriocin, so it is capable of elaborating bacteriocins that are effective only at an acid pH.12 This principle is illustrated in the following case study (Fig. 1).

In evaluating the possible qualitative and quantitative effects of the menstrual cycle on the bacterial flora of the female genital tract, serial posterior vaginal pool cultures were obtained from C. N., who had had "chronic candidiasis" intermittently for the previous 2 years and had come to accept this fact as a way of life. The repeated absence of any anaerobic bacteria came as a minor shock. Gorbach et al.,18 among others, have demonstrated the almost universal presence of anaerobic bacteria as constituents of the bacterial flora of the female genital tract. Confirmation of the initial observation, coupled with the relative simplicity of the qualitative bacteriologic findings, suggested regulation by bacterial interference. With the use of an agar overlay technique (in which the strain of C. albicans is isolated and grown in vitro; the colonies are removed and de-

stroyed by both mechanical and physiochemical means; the agar nutrients are replenished by overlaying with new agar; and the aerobic or anaerobic bacteria common to the female genital tract are plated onto the agar), it could be shown that where the colonies of C. albicans had been, the growth of selected challenge bacteria was inhibited.14 With the use of a flip agar technique (in which the agar, after growth and removal of the Candida colonies, is turned over and the opposite side plated with the challenge organism), it was demonstrated that the substance that caused inhibition of growth had the capability of diffusing through the agar.16 This observation made the probability reasonably good that the phenomenon observed was mediated through a bacteriocin and not by a local change induced by C. albicans in the agar.

Following therapy, anaerobic bacteria became part of the patient's vaginal flora.

Vulvovaginal candidiasis (genesis IIB: Ping-Pong vaginitis). Ping-Pong vaginitis is a variant antibiotic-induced candidiasis in which antimicrobial therapy is given for antecedent genital tract infections, rather than for infections involving another organ system.

The classic history of Ping-Pong vaginitis is that of the patient doing significantly better for a short period of time and then becoming worse. A carefully taken history indicates a change in the symptom complex (e.g., appearance of itching, change in character of discharge). If the physician assumes that there has been a simple therapeutic failure and therefore does not completely reevaluate the patient (which includes doing the potassium hydroxide and wet-mount preparation and sometimes cultures), the correct diagnosis may be

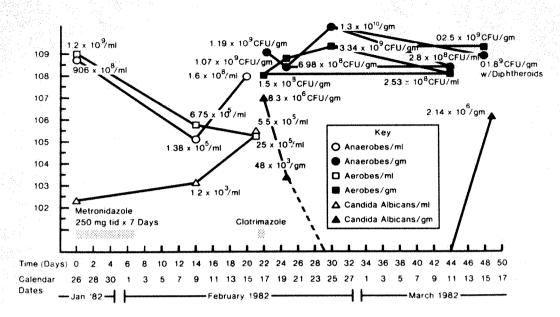


Fig. 2. Vulvovaginitis case study (W. P.): secondary *C. albicans* vulvovaginitis induced by metronidazole therapy for *Gardnerella* vaginitis.

missed. The physician may then prescribe a generally different, but pharmacologically similar, drug that may even aggravate the situation. One particularly unfortunate patient was a middle-aged woman who developed primary candidiasis. She responded to initial therapy, only to return 5 days after termination because her vaginitis suddenly became worse. She was finally referred for recurrent vaginitis after five courses of anticandidal therapy. At the time we saw her, she had an allergic vulvitis. In retrospect, her pruritus had ceased following therapy, but she had subsequently developed an increased vaginal discharge that was associated with an unacceptable odor. In the course of a therapeutic study using metronidazole for G. vaginalis, a classic case of IIB vulvovaginal candidiasis developed (Fig. 2).

The patient had a characteristic case, clinically, of "nonspecific vaginitis" confirmed by both qualitative and quantitative microbiologic studies. Not only was G. vaginalis isolated, but quantitatively it was the dominant aerobic bacterium. This patient had an insignificant number of colony-forming units of C. albicans per gram of vaginal fluid. The patient was given 250 mg of metronidazole three times a day for 7 days, which effectively eliminated G. vaginalis. With the resultant fall in the number of colony-forming units of anaerobic bacteria per gram of vaginal fluid, there was a progressive increase in the number of colony-forming units of C. albicans. Fifteen days after therapy, the patient developed overt vulvovaginal candidiasis. The delay in seeking therapy was in part related to the intervention of a menstrual period, which afforded some symptomatic relief. With clotrimazole therapy (a single 500 mg intravaginal tablet), both a clinical and a microbiologic cure was achieved. Twenty-four hours after insertion, the colony count had dropped from 8.3×10^6 to 4.8×10^3 CFU/gm of vaginal fluid. When sampled again at 5 and at 20 days after therapy, *C. albicans* was below the threshold of detection (10^2 CFU/gm of vaginal fluid). Twenty-four days after therapy, *C. albicans* again appeared as a constituent of this patient's vaginal flora. Despite its presence, the patient continued to be asymptomatic.

In antibiotic-induced disease, the major goal is the eradication of the vaginal reservoir of infection. If the vaginal reservoir is eliminated, unless a concomitantly significant vulvitis is present, the problem will be cured. Preference is given to the tablet form of therapy because of better patient compliance. The creams are messy and psychologically less acceptable to a significant number of women.

Clotrimazole tablets are highly effective in antibioticinduced vulvovaginal candidiasis, whereas the cream applied intravaginally and to the perineum appears to be more efficacious in those cases of idiopathic primary vulvovaginal candidiasis characterized by significant vulvitis.

Vulvovaginal candidiasis (genesis III: Systemically influenced candidiasis). The critical factor selecting for systemically induced candidiasis is an increase in the glucose substrate available in the local microbiologic environment or T-cell dysfunction. For patients with systemically induced vulvovaginal candidiasis caused by steroids, diabetes mellitus, or immunodeficiency involving T-cell function, intravaginal and/or vulvar therapy with topically applied anticandidal creams and

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with oral ketoconazole is preferred. It is imperative to treat the underlying condition aggressively. Candidiasis associated with poorly controlled diabetes will recur unless carbohydrate metabolism is brought under control.

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Mode of action of clotrimazole: Implications for therapy

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Ergosterol is an essential constituent of the fungal cytoplasmic membrane. Clotrimazole and other azoles interfere with the ergosterol biosynthesis in a concentration-dependent fashion. Although low concentrations exhibit only a partially inhibitory effect, high concentrations may completely block ergosterol synthesis. Reduction of fungal growth and inhibition of growth and fungicidal action during prolonged incubation are the corresponding effects at the cellular level that are a consequence of ergosterol depletion. The inoculum effect, the influence of the incubation period, and the influence of nutrient media, three factors that often complicate susceptibility testing in vitro, can also be explained by the mode of action of azole compounds. Another interesting characteristic of azole antifungals was revealed by the observation that hyphae and pseudomycelia of *Candida albicans* are much more susceptible to azoles than are yeast cells. Even 1% of the minimum inhibitory concentration of clotrimazole may totally inhibit mycelial growth in vitro. This may be of clinical importance, since germination was reported to enhance adherence of *C. albicans* to buccal and vaginal epithelial cells. (AM J OBSTET GYNECOL 1985;152:939.)

Key words: Candida albicans, clotrimazole mode of action, azole antifungals, therapy

Clotrimazole (Fig. 1) was the first imidazole derivative developed for treatment of human mycotic infections. It was synthesized at the Bayer research laboratories and first described in 1969. The azoles now play an essential role in antifungal therapy; research on new products is being conducted by pharmaceutical companies all over the world.

From Bayer AG, Institut für Chemotherapie.

The general chemical structure of imidazolyl antimycotics is depicted in Fig. 2. From the screening of thousands of discrete structures, we know that two features are required for bioactivity: the unsubstituted imidazole (or triazole) ring and the covalent N-C (nitrogen-carbon) bond between the heterocyclic ring and the remainder of the molecule. The other substituents linked to the central carbon atom influence pharmacologic properties to a greater extent than antifungal activity.²

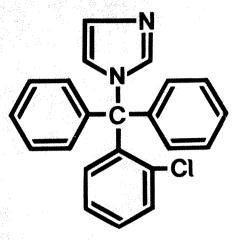


Fig. 1. Chemical structure of clotrimazole.

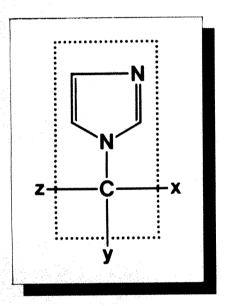


Fig. 2. General chemical structure of imidazolyl antifungals.

Some important group-specific properties of the azole antifungals are listed in Table I. They influence not only the outcome of in vitro susceptibility tests but also the therapeutic effect to be expected in vivo. The molecular mechanism of action is the major key to a better understanding of the specific type of antifungal activity, as exhibited by the azole compounds.

It has been suggested^{3, 4} that unsaturated fatty acid components contained in the plasma membrane might be the target site of imidazole action. In fact, a direct interaction of azoles with membrane constituents could explain the antibacterial effect, as well as the residual activity exhibited against nonproliferating fungal cells. On the other hand, there is strong experimental evidence that inhibition of the sterol biosynthesis is the major mechanism of antifungal action.^{5, 6} Fig. 3 schematically shows the biosynthesis of ergosterol, which is

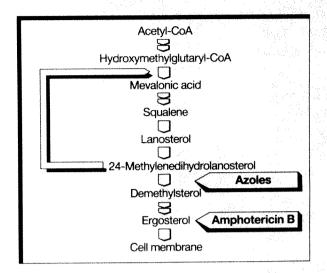


Fig. 3. Suggested mechanism of action of imidazolyl antifungals. CoA = Coenzyme A.

Table I. Characteristics of antifungal activity as exhibited by azole compounds

- 1. Broad spectrum of antifungal activity
- 2. Concentration-dependent scale of antifungal effects
- 3. Lag phase
- Dependence on test conditions in vitro (inoculum, medium, incubation period)
- 5. Dependence on physiologic condition of fungus
- 6. No resistance development

an essential and integral part of the fungal cytoplasmic membrane. Clotrimazole and other azoles were found to inhibit specifically the demethylation of 24-methylenedihydrolanosterol, which then accumulates, leading to an additional feedback control of the whole pathway. Gradual ergosterol depletion of the cytoplasmic membrane is considered to result in alterations of the membrane structure and to lead finally to a lethal efflux of ions and cytoplasmic material.

Some electron microscopic pictures may illustrate the morphologic changes in the gross cell wall. Samples of vaginal smear containing *Candida albicans* were taken from patients treated with a 100 mg clotrimazole vaginal suppository. Three hours after therapy, appendices of the cytoplasmic membrane and cytoplasmic areas of lower density could be seen (Fig. 4, A). After 6 hours, the cytoplasmic membrane was partially disrupted (upper part of Fig. 4, B) and vacuoles had formed. Three hours later, lysis of the fungal cytoplasma had occurred (Fig. 4, C).

Several of the properties of azoles, as outlined in Table I, can readily be understood as consequences of the suggested mechanism of action. The broad spectrum of activity is in agreement with the finding that all fungi contain ergosterol as a characteristic cell wall

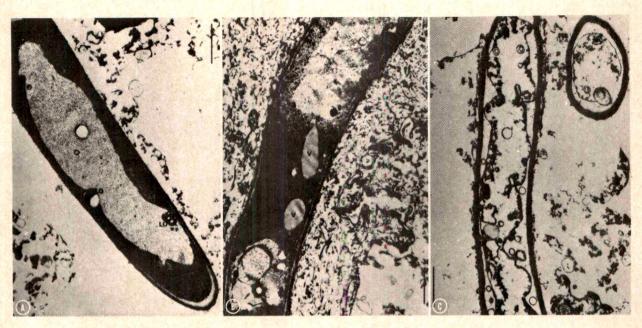


Fig. 4. Electron microscopic pictures of C. albicans cells in vaginal smear of patients treated with clotrimazole. Samples were taken 3 hours (A), 6 hours (B), and 9 hours (C) after therapy.

Table II. Minimum inhibitory concentrations (mg/L) of clotrimazole against C. albicans as measured by broth dilution method under various test conditions*

	Incubation period		
Inoculum	2 days	3 day:	
10 ³ cells/ml	0.25	1	
	(0.062)	(0.5)	
105 cells/ml	32	32	
	(4)	(4)	

^{*}Concentrations in parentheses indicate 90% to 95% inhibition.

constituent. The lag phase, which refers to the period of time required between the addition of drug and the onset of growth inhibition, is due to the fact that existing pools of ergosterol have to be depleted before the cell wall structure is affected. Growing organisms are much more susceptible than resting cells to azoles because growing organisms need ergosterol for incorporation into the areas of newly synthesized cell wall material. The concentration-dependent range of antifungal effects (reduction of fungal growth at subinhibitory concentrations, inhibition of growth at the minimum inhibitory concentration, and fungicidal activity at 5 to 10 times the minimum inhibitory concentration) is caused by the fact that inhibition of the sterol synthesis itself is a concentration-dependent process. Low concentrations of clotrimazole partially inhibit

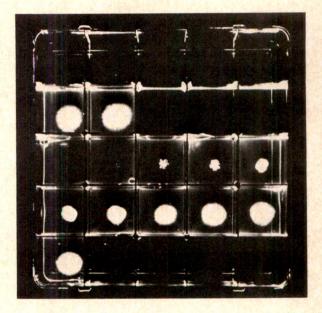


Fig. 5. Agar dilution with clotrimazole, with Microsporum felineum used as the test organism. Two growth controls (top left) were used and then 64-32-...-0.062 mg/L of clotrimazole. The inoculum used was about 2 × 10² fungus particles per plot.

sterol biosynthesis; as the drug concentration is increased, there is an increase in the degree of sterol synthesis until, finally, the biosynthetic pathway is blocked completely above a certain concentration.

Fig. 5 gives an example of the gradually increasing inhibitory effect of clotrimazole against dermatophytes.

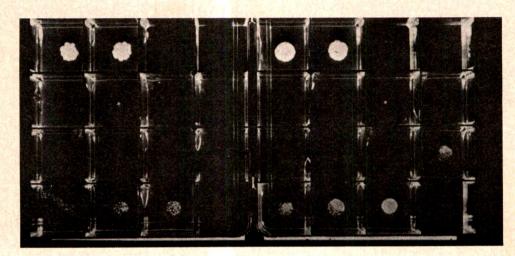


Fig. 6. Agar dilution test with clotrimazole, with *C. albicans* used as the test organism. On each plate, two growth controls (top left) and then 64-32-...-0.062 mg/L of clotrimazole were used. The inocula were 10³ cells per plot (left) and 10⁵ cells per plot (right).

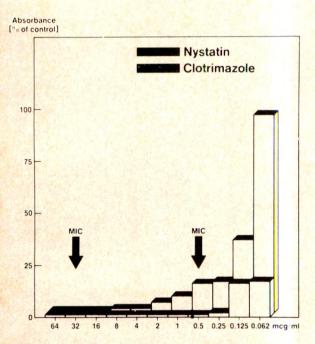


Fig. 7. Cell density of *C. albicans* in the broth dilution test at various concentrations of clotrimazole and nystatin. Samples were measured turbidimetrically, and the absorbance was plotted in percent of the growth control. The *arrows* indicate the conventional minimum inhibitory concentrations as read visually. *MIC*, Minimum inhibitory concentration.

As depicted in Fig. 6, the same wide range of partial inhibition can also be seen with yeasts. It should be mentioned that on the left agar plate, which was inoculated with a smaller inoculum than the right one, the fungal growth rate at the lowest concentration tested was still reduced considerably in comparison with the growth controls.

Fig. 7 shows the results of a broth dilution experi-

Table III. Influence of inoculum size on azole susceptibility tests

Initial inoculum	Final cell count	Result
Control		
10 ⁴ /ml	10 ⁷ /ml	Visible growth
10 ⁶ /ml	>10 ⁸ /ml	Visible growth
95%-99% inhibition		
10 ⁴ /ml	$1-5 \times 10^5/\text{ml}$	Not visible
10 ⁶ /ml	$1-5 \times 10^7/\text{ml}$	Visible growth

ment performed with clotrimazole and nystatin. The fungal density obtained after 24 hours of incubation was measured turbidimetrically; the arrows indicate the minimum inhibitory concentrations as read visually. It can be seen that clotrimazole considerably reduced fungal growth even at concentrations far below the minimum inhibitory concentration, whereas the inhibitory effect of nystatin ended rather abruptly, as soon as the concentration dropped below the minimum inhibitory level. Thus a comparison of the inhibitory effects of azoles at very low drug concentrations can lead to an assessment that differs completely from the one made on the basis of conventional minimum inhibitory concentration values. This difference may be of clinical importance with regard to the concentrations to be expected in tissue after oral treatment or in the deeper layers of skin or mucosa after topical application.

What is the reason for the influence that inoculum and incubation period have on the results of susceptibility tests? The minimum inhibitory concentrations

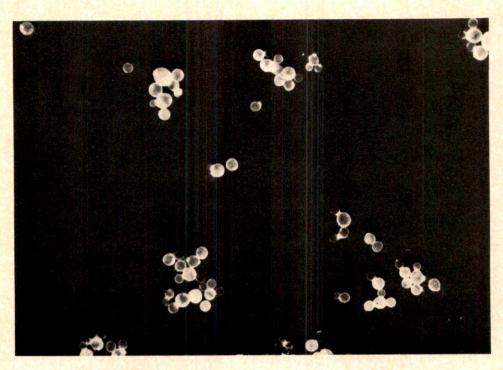


Fig. 8. Budding of C. albicans yeast cells in Sabouraud medium.

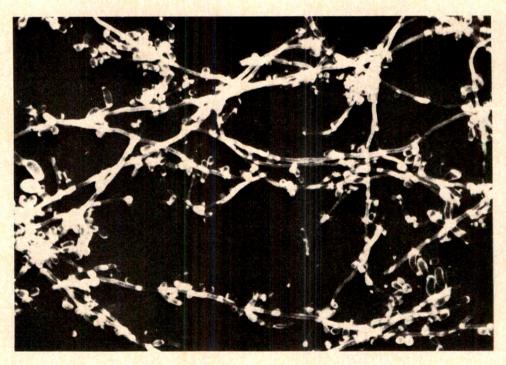


Fig. 9. Mycelial growth of C. albicans in Eagle medium.

shown in Table II may illustrate this problem, which has caused a lot of confusion in the past. One explanation is that under unsuitable test conditions, a small rate of residual growth may lead to a discernible sediment of fungus in the test tube or to a haze of growth on the agar surface.8,9 The data summarized in Table

III prove that even when fungal growth is inhibited by 95% or more in comparison with control tests, an inoculum of 105 to 106 yeast cells per milliliter will result in clearly visible growth.

One last aspect may be of clinical importance. Under in vivo conditions of infection, C. albicans grows pref-

Table IV. Minimum inhibitory concentrations of clotrimazole on *C. albicans* mycelia and yeast cells

Medium	Growth phase	MIC (mg/L)
Sabouraud medium	Yeast cells	16-32
Eagle medium	>70% mycelia	0.125

MIC = Minimum inhibitory concentration.

Table V. Solubility of clotrimazole in 0.1 molar lactic acid–lactate buffer system

рН	Solubility (mg/dl)	
6.0	0.3	
5.0	0.6	
4.0	5.7	
3.0	36.8	

erentially as pseudomycelia, whereas in usual nutrient media the yeast cells multiply by budding (Fig. 8). However, mycelial growth can also be induced in vitro by using tissue culture media such as Eagle medium (Fig. 9). When the inhibitory effects of clotrimazole in Sabouraud and Eagle media were compared, the mycelia proved to be about 100 times more susceptible than were yeast cells. 10 This effect is shown in Table IV. Sobel et al.11.12 reported that germination of Candida yeast cells enhanced the adherence to buccal and vaginal epithelial cells and that the addition of azoles to a germination-promoting medium not only inhibited mycelial growth but also considerably reduced the rate of fungal adherence. Moreover, it was found that C. albicans adherence to vaginal cells was considerably greater at pH 6 than at pH 3 to 4. The acid formulation of the clotrimazole vaginal tablet may thus reduce fungal adherence and support the eradication of the fungus from the site of infection in two ways: (1) by specifically inhibiting germination and mycelial growth and (2) by restoring the physiologic acid pH of the vagina.

Within a wide range of concentrations, a rise in the amount of clotrimazole will lead to increased activity. The transition from purely fungistatic to fungicidal action is considered to be especially important in this respect. For a long time it seemed, however, that the low water solubility of clotrimazole would be a physi-

cochemical limitation that could not be overcome. Table V shows the solubility of clotrimazole as measured in a 0.1 molar lactic acid—lactate buffer system at different pH values (Volkman D. Data on file, 1983 Bayer AG, Wuppertal, Federal Republic of Germany). It is obvious that solubility is increased dramatically at pH 4 and pH 3, in comparison with neutral pH. This increase in solubility was the major reason to develop an acid formulation of clotrimazole for treatment of vaginal *Candida* and *Torulopsis* infections, and the lactic acid—lactate buffer system is of course very much in agreement with the physiologic conditions of the vagina.

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Pharmacokinetic fundamentals of vaginal treatment with clotrimazole

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Pharmacokinetic studies with clotrimazole in rats and in humans, following ora and intravenous administration, have shown that clotrimazole is rapidly metabolized. After vaginal treatment with clotrimazole, the small fraction absorbed into the systemic circulation—between 3% and 10% of the dose—is subjected to metabolism and excretion as after oral or intravenous administration. The vaginal availability of clotrimazole is largely dependent on the formulation applied. In contrast, up to 3 days after single application of a vaginal tablet containing 500 mg clotrimazole together with lactic acid, fungicidal amounts of clotrimazole were measured in vaginal fluid, i.e., the single dose serves as a depot in the vagina for at least 3 days. Thus the single-dose treatment of vaginal mycosis with clotrimazole offers the advantage of combining a high availability in the vagina with a low availability of systemic circulation and is a means of solving the problem of the patient's noncompliance. (AM J OBSTE—GYNECOL 1985;152:945.)

Key words: Clotrimazole, pharmacokinetics, vaginal treatment

Once a drug has entered the systemic circulation, the same principles for distribution, metabolism, and excretion have to be applied as for oral or intravenous administration. Therefore, for an understanding of the fate of clotrimazole after vaginal application, the pharmacokinetics after oral and after intravenous administration will be presented.

Results and comments

A great number of pharmacokinetic studies in animals and in humans have shown that clotrimazole is rapidly metabolized by the liver. After oral administration, clotrimazole is nearly completely absorbed. Related to a single oral dose of 500 mg of radioactively labeled clotrimazole, peak concentrations of radioactivity (equivalent to about 5 µg clotrimazole per milliliter of serum) were reached (Fig. 1). However, only about 1% of the radioactivity in the serum represents unchanged cletrimazole, as was determined by a specific thin-layer chromatographic method.1 Thus nearly the entire radioactivity in the circulating blood could be attributed to metabolites, three of which have been found in serum. In the urine of volunteers receiving clotrimazole orally, two major metabolites and three minor metabolites have been detected and identified. These metabolites are microbiologically inactive.

The results of the studies with radioactively labeled clotrimazole clearly indicate that the low concentrations of unchanged clotrimazole in the blood are due to a pronounced first-pass metabolism by the liver. It has to

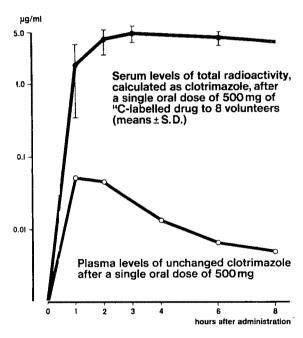


Fig. 1. Absorption of clotrimazole after oral administration.

be emphasized that the rapid and extensive hepatic clearance also holds true for clotrimazole absorbed from the vagina.

For an estimation of the absorption of clotrimazole following vaginal application, the serum levels of radioactivity were determined in female volunteers who received 100 mg of labeled clotrimazole either as a vaginal taplet or as vaginal cream 1% (Fig. 2). By comparison with the data obtained after oral administra-

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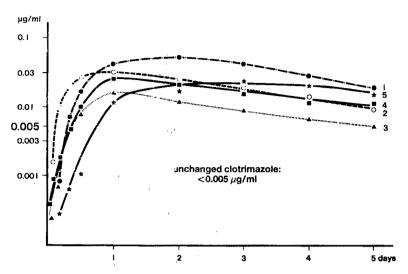


Fig. 2. Serum levels of radioactivity, calculated as clotrimazole, after vaginal application of 100 mg as a tablet. (From Ritter W, Patzschke Z, Krause U, Stettendorf S. Pharmacokinetic fundamentals of vaginal treatment with clotrimazole. Chemotherapy 1982;28[Suppl 1]:37, published by S. Karger AG, Basel.)

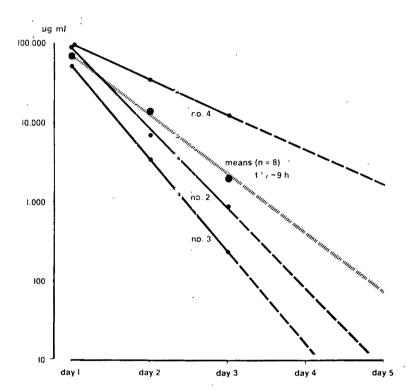


Fig. 3. Amounts of clotrimazole ir vaginal fluid after a vaginal dose of 500 mg.

tion, the fraction of this dose reaching systemic crculation after vaginal application was estimated to be between 3% and 10%. However, an independent study with six volunteers revealed that the plasma leves of unchanged clotrimazole were lower than 0.005 µg/ml.

Obviously, the aim of an efficient local vaginal trerapy for vaginal mycosis is to reach a high concentration of the drug at the site of infection and, at the same time, to have low blood levels to avoid systemic side

effects and the interactions with other drugs given concomitantly. The single-dose therapy with 500 mg of clotrimazole was introduced to fulfill these requirements and to solve the problem of the patient's noncompliance, encountered with the 6-day and even with the 3-day treatment schedule.

A new formulation containing 500 mg of clotrimazole together with lactic acid was studied in volunteers with regard to vaginal availability and to the concen-

Table I. Amounts of clotrimazole in vaginal fluid after a single vaginal dose of 500 mg

77 - I A	Clotrim	azole in vaginal (μg/ml)	! fluid
Volunteer No.	24 hr	48 hr	72 hr
1	49,000	100	<15
2	90,000	6,800	910
3	51,000	3,500	240
4	91,000	36,500	13,230
5	12,000	900	<20
6	152,000	62,200	1,910
7	36,000	2,600	30
8	64,000	500	<75

Modified from Ritter W, Patzschke K, Krause U, Stettendorf S. Pharmacokinetic fundamentals of vaginal treatment with clotrimazole. Chemotherapy 1982;28(Suppl 1):37, published by S. Karger AG, Basel.

trations of unchanged clotrimazole in the blood plasma for up to 3 days after application. Since tolerance was investigated as well, this study was performed on a double-blind basis with placebo as the comparison.

Twenty-four hours after the application of a 500 mg vaginal tablet, amounts of clotrimazole between 12 and 152 mg/ml were found as a suspension in vaginal fluid. Even after 48 hours, milligram quantities of the active drug were measured in vaginal fluid, and after 72 hours, fungicidal concentrations of clotrimazole were still detectable (Table I).

These results indicate that the single-dose treatment is actually a 3-day treatment but has the advantage that patient noncompliance is avoided. In addition, overcompliance is avoided as well: since there is only one tablet, the patient cannot treat herself with two tablets simultaneously.

In contrast to the high levels of clotrimazole in vag-

inal fluid, the concentrations in the blood plasma of the volunteers were lower than 0.01 µg/ml at any time up to 72 hours, which demonstrates that the ultimate goal of a simple, safe, and reliable treatment of vaginal mycosis was realized: a high concentration at the site of action and, at the same time, very low drug levels in the circulating blood.

For a demonstration of the pharmacokinetic advantages of the new lactic acid-containing formulation for the 1-day treatment, clotrimazole concentrations in vaginal fluid after the third dose of 200 mg used for the 3-day treatment were compared with those after the single-dose treatment with 500 mg. Whereas the drug amount of the new single-dose tablet was increased by a factor of only 2.5%, the vaginal availability of the 500 mg tablet was apparently increased—because of the lactic acid—by a factor of 50 to 500. The decline of the mean clotrimazole amounts in vaginal fluid during the single-dose treatment seems to be a first-order process, with a mean vaginal clotrimazole half-life of about 9 hours. If we extrapolate the concentration decay in vaginal fluid, we are able to predict fungicidal clotrimazole concentrations—after application of a single vaginal dose of 500 mg-for at least 4 or 5 days (Fig. 3).

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The problem of patient compliance

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The problem of noncompliance is an increasing and varing one. Major reasons for this dilemma include a growing skepticism concerning the ability and dedication of the health care industry, as well as a growing population of older people. A review of medical history reveals ancient attitudes toward compliance. There are special problems for special people, such as infare, phildren, adolescents, elderly persons, and those who are hostile or have a "sick" wish or "death" wish. Persons who require long-term therapy require special care. Child abuse and abuse of elderly persons may be involved. Problems of decreased visual acuity, color perception, and deafness must be dealt varth on an individual basis. The responsibility of the physician, which includes nonobtrusive monitoring, is arcesed. Consideration is given to the pharmaceutical industry and the role it can play to help mitigate the problem. In conclusion, anticipation of the problem and improved doctor-patient relationships will achieve desired results. (AM J OBSTET GYNECOL 1985;152:948.)

Key words: Patient noncompliance, health care inclustry

Since adequate therapy is dependent on patient compliance, it is surprising that little attention was paid to this important subject until approximately 15 years ago. A perusal of the medical literature since the erriest times indicates that there is little, if any, reference to compliance (Adams L, personal communication. May 1, 1983). In recent years, however, more attention has been paid to this problem, and some reasons for this include increased longevity; long-term, often lifetime, therapy; and the necessity for multiple and more complicated regimens.

It is of interest that recent editions of such well-known volumes as *The Pharmacologic Basic of The apeutics*, by Goodman and Gilman, and Dr. Tinsley Harrison's *Harrison's Principles of Internal Medicine* bot contain sections on compliance. I have not found, however, that this subject is stressed in the standard gynec alegic text.

If the ancients considered compliance at all, they left sparse records of it. Many societies, not necessarily primitive ones, considered illness as punishment inflicted by the gods for sins committed, and exorcing of pathologic demons was sometimes the only theraget tic attempt to restore health. Compliance apparents depended on the devoutness of the sufferer.

In ancient primitive societies such as tribal Africa and New Guinea, witchcraft and sorcery played an important role in treatment. This is true, even today. Riualistic frenzies were carried out in order to identify the enemy responsible for the sufferer's malady. Even though these attitudes may have predominated, ht man beings have always sought to help themselves. Much of the primitive medicine was herbal in nature; some was

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efficacious and some totally ineffective. Superstition, shamanism, fakery, and quackery were obstacles to deal with then, as they are today.

Scope of the problem

Even in today's enlightened society, it has been estimated that only one third of prescriptions written are ever filled, one third of the patients obtain the medication but do not adequately comply with the dosage regimen, and only one third take the medicine as prescribed.

In a study involving outpatients treated at a university clinic, Latiolois and Berry³ found that 77 of 180 patients (42.8%) misuse their medication. They either overdose or underdose themselves. When they overdose, they take more medication than was prescribed at one time or more in any given day, or they take it at the wrong time. When they underdose, they usually take less than was prescribed at any given time, omit a dose, or take it at the wrong time of day or by the wrong route of administration. They may discontinue medication or take outdated medication. They may take someone else's medication, take a combination of drugs that are incompatible, or just fail to have the prescription filled.

Compliance and public health

Probably the first emphasis noted in the medical literature concerning any sort of compliance was related to public health measures. Compliance was dependent on the government of the country and involved water purification, sewage disposal, pure food laws, compulsory inoculation, isolation of persons with infectious disease, and sc forth. The saga of Typhoid Mary in the United States vividly illustrates the problems encountered. Presently there is an effort to persuade persons

at high risk for acquired immune deficiency syndrome to refrain from donating blood. According to blood bank personnel in the American Red Cross, this compliance seems to be working rather well. Our campaigns against alcoholism and narcotic addiction have been helpful but less successful. Possibly the best compliance is seen with patients whose symptoms indicate the need for medication, that is, symptom-triggered compliance. Classic examples of patients complying in this way include patients with anginal pain who take nitroglycerin and those afflicted with gastrointestinal symptoms or migraine headaches. In female patients, vulvovaginitis caused by Candida usually leads to compliance. The triggering symptom is usually pruritus, and it is often said that itching is worse than pain.

Responsibility of the physician: Establishing a good physician-patient relationship

Possibly the largest share of the problem lies with the physician. It appears that some physicians think their Aesculapian authority will ensure compliance and that once a prescription is written, their principal responsibility has been fulfilled. This is, in my view, a Panglossian idea at best. The patient, on the other hand, may interpret the mystical symbols on a small piece of paper to be a Cassandra-like forecast of doom.

The medical profession must drop the mantle of scientific chauvinism and communicate with every patient in human terms. Explanations should be explicit and in understandable language. A thorough review of the perceived diagnosis, the reason for the medicine prescribed, how it will look, how it will taste, what it is expected to do, and the possible side effects must all be covered.

Furthermore, it is the responsibility of the physician to identify those patients likely to be poor compliers. Some categories of patients tend to be poor compliers, such as adolescents, elderly persons, patients living alone, hostile patients, and those who relish the "sick role" and do not really want a cure. John Steinbeck⁴ eloquently described the latter type in his novel East of Eden: "Some people think it is an insult to the glory of their sickness to get well." It is always necessary to emphasize the importance of the proposed treatment. This is particularly important in asymptomatic disease such as essential hypertension. Some patients think it smart to thwart the physician. To paraphrase Mark Seigler, professor of medicine at the University of Chicago, Pritzker School of Medicine, they have the counterfeit courage of the noncompliant. Often it is helpful to enlist the assistance of others, such as the family, friends, nurses, and home care facilities. It should be noted that the simpler and shorter the therapeutic regimen, the better the compliance. For example, in the treatment of vulvovaginal candidiasis, compliance is better with a 3-day course of treatment than a 7- or 11day one. The new single-dose treatment with the 500 mg clotrimazole tablet (Mycelex G) ensures virtually 100% compliance.5-10

Responsibility of others

In the care of the sick child, the parents are usually the ones who must be responsible, and the period of illness may be a trying time for all. It is no wonder that pediatricians frequently resort to parenteral therapy. Nurses have the responsibility for hospitalized patients. There are certainly problems involved in health care facilities, whether they be hospitals, long-term care facilities, clinics, or home nursing or home visiting programs.

Medication errors, when discovered by the patient, destroy confidence and inhibit compliance. A recent article by Harry Henderson¹¹ in Medical Tribune cited an Auburn University study directed by Dr. Kenneth Barker. The results revealed that only 31% of longterm care facilities and 40% of hospitals passed a medication error limit of 6%. In 58 large hospitals and 10 small hospitals, the medication error rate was 12% and 11%, respectively. The highest nursing home rate was 30% and the lowest 0%, an indication that there are some good nursing homes. I wish to state that this study was not meant to be an indictment of the nursing profession. Nurses certainly have their problems. Having recently been a patient myself, I agreed with Dr. Lewis Thomas¹² when he said, "I am on the nurses' side." Physicians' orders are often illegible and occasionally wrong. Charles D'Orleans (1394-1465) forced his physicians to write their prescriptions in verse; the additional time, thought, and care must have ensured accuracy. I never resent a nurse's calling me to verify an order. In the same vein, I appreciate it when a pharmacist finds a prescription to be confusing and calls to confirm the medication or directions.

Role of the pharmacist

The important role of the pharmacist was addressed by Ford18 in an article entitled "Apothecaries' Role Reassessed." Pharmacists often know of other medications the patient is taking, including some incompatible medications and some over-the-counter medications. Their role in facilitating quality care and compliance is enormous.

Preventive medicine and compliance

The physician wishing to use prophylactic medication may have to contend with special problems. For example, estrogen is prescribed to retard development of osteoporosis, but the therapist may be met with patient reluctance because of fears of perceived dangers. Other examples of treatments that cause fear include the use

of perioperative blood transfusion for the treatment of severe anemia or excessive blood loss, the use of preoperative antibiotics, the prophylactic use of an.:b.otics in patients with valvular heart disease, and the rreatment of asymptomatic diseases, morbid obes ±y, and even dysplasia of the cervix uteri. Dietary programs and exercise programs are notoriously neglecte L Mark Twain once said that he occasionally felt an im zu se to exercise, but if he would lie down, this feeling would pass. Patients are frequently cautioned to avoi: tress as much as possible—a difficult feat at best and Trually impossible in today's world. High-risk personnel are often reluctant to receive prophylaxis against hepatitis. Immunizations, whether for childhood diseases or for protection against rabies or tetanus, continue to present a problem of compliance.

In spite of the new "state of the art" equipment, many patients reject mammography screening because of fear of radiation. They also fear chest x-ray elaminations, computerized axial tomography, angiography, etc. Norman Cousins held out in his determination to avoid coronary angiography following his recent nearly fatal myocardial infarction, and his feelings were eloquently portrayed in his new book, The Healin-Heart.

Policing

Assessment of the degree of compliance recuires some monitoring and must be carried out in an unobtrusive manner to be effective. Simple measures, such as family surveillance, home visits, and repeated explanations concerning each drug regimen, are all important. One must avoid the "Hawthorne effer" so as not to stigmatize the patients, as Hester Pryune was stigmatized in the Scarlet Letter. The testing of serum blood levels may at times be necessary.

It is important that the physician's approach be friendly and open, emphasizing the importance frompliance in language that is understood by the patient. Aesculapian authority must be dropped and the patient-physician relationship must be a cooperate one rather than an adversary one. The physician result not pretend to be "Big Brother," as depicted in George Orwell's 1984. It is amazing to reflect that the book was written in 1949, and 1984 is here.

It may be helpful to label bottles in simple terms, such as "This is a water pill," "This is the heret pill," or "This is the pressure pill."

Pharmaceutical industry and compliance

Drug companies have diligently sought to implify treatment regimens by producing long-acting medication, transdermal medication, symptom-toggered medication, intradermal pellets, insulin pump spacetabs, pacemakers, and short-term treatment regimens. In addition, the osmotic pump capsule may soon be

perfected. Some problems are solved with these approaches; however, the physician must still evaluate the individual patient, including idiosyncrasies and tolerance. This problem was eloquently addressed in an article by Sanford Roth entitled "The New Look in Drugs: Is Longer-acting Better?"17 Industries are caught between the Scylla of effectiveness and the Charybdis of safety. Human experiments are difficult and sometimes dangerous, and animal rights groups are challenging the use of animals in drug evaluations. To one involved in clinical research, it is interesting to read the words of Roger Bacon, 18 written in the thirteenth century: "Scientists in other fields can experiment on inanimate objects and test their results until all faults and errors are eliminated, but doctors are not able to do this because of the dignity and superiority of the material they're dealing with, and since truth cannot be reached except by experimentation, perhaps doctors should be excused if they make more mistakes than other experimental scientists."

Additionally, the pharmaceutical industry has initiated other attempts to help with the compliance problem, including improvements in packaging, color, taste, and appearance of medications. It is important to consider the special problems of certain patient groups. For example, elderly persons have diminution of the senses. In addition, as pointed out previously, safety caps may easily be opened by the persistent child but may present an insurmountable obstacle to the elderly patient. The drug vendors are aware of these problems and do an excellent job in addressing them.

It is also incumbent on the pharmaceutical industry to conduct long-term assessments concerning drug safety and efficacy. The prescription event monitoring in the United Kingdom has taken a lead in this direction. It is hoped that physicians will give wholehearted support to the manufacturers. The Bayesian statistical method of continuous reevaluation of data is essential, and cooperation between practitioner and manufacturer is mandatory.

Special problems in obstetrics

Because of the thalidomide tragedy and the sad saga of diethylstilbestrol, obstetrics patients are understandably wary of medications. Public hysteria and consumer advocates have caused the withdrawal of at least one safe and effective drug, Bendectin.

It seems strage that some women are less willing to take necessary medications than to give up alcohol and nicotine. Because of the special problems during pregnancy, the obstetrician may find himself walking a tightrope—a situation that Dr. Randall Morgan calls "nuisances versus nuances" (personal communication, June 1984).

If one consults the Physicians' Desk Reference or the

package insert, the usual caveat is: "The safety of this drug during pregnancy has not been established." A physician is exhorted to weigh the possible benefits against the risks. At times this is an awesome responsibility. Clinicians would like to see more primate studies using appropriate dosage regimens.

The media and compliance

Media coverage of medical misadventures, as well as of medical miracles, influences attitudes. Recent books, such as Pills That Don't Work, by Dr. Sidney Wolfe,19 have created much doubt in the minds of patients concerning drugs.

The problem of the media and medicine was recently addressed by author Joan Goodfield20 in her book Reflections on Science and the Media: Are the media fair to science? Is science fair to the media? Sensational reporting may sell newspapers but often does a disservice to the health care industry. Scandals such as the Sloan-Kettering fraud, detailed in the best-selling book The Patchwork Mouse,21 and the Darcy affair at Harvard have made medical ethics a major issue.

Animal rights groups have castigated investigators and have made the procurement of animals more difficult. One must admit that some supervision of animal care and avoidance of unnecessary suffering are needed.

Serious drug reactions and large damage awards have not been slighted by the press and have served to erode public trust. The problem was addressed in the Medico-Pharmaceutical Forum in the United Kingdom. A synopsis was reported in the Journal of the Royal Society of Medicine. 22 The tremendous power of the press and its responsibility were discussed. Although there was an understandable lack of accord, it was pointed out that the media frequently failed to follow up when later evidence gave them greater insight. They were exhorted to correct previous excesses. The greatest stumbling block in the United Kingdom, as well as in the United States, seems to be a mutual lack of confidence.

Compliance and medical curriculum

Very little formal attention is given to the subject of compliance in most medical schools, although I am sure that it surfaces with some frequency in the clinical setting.

Involvement of governmental agencies and professional organizations

The enormity of compliance problems has concerned the bureaucracy. The Food and Drug Administration sent patient education brochures to 36 million social security recipients with their checks. This effort was in response to a memorandum sent to the agency's board

by then Commissioner Dr. Arthur Hull Hayes. The memo states: "I believe we now need to take a more aggressive stand and pursue additional activities to insure that the patient achieves his patient education goals."

The brochure advised patients to learn all they could concerning prescription drugs. That information included compatibility with food, drink, and other drugs. Other advise included learning about side effects, how and when to take medication, and when to stop. Many professional organizations have inaugurated patient information projects, including the American Medical Association, the American College of Obstetricians and Gynecologists, the American College of Surgeons, the American Academy of Family Physicians, and the National Association of Retail Druggists. In addition, the pharmaceutical industry has initiated patient education programs.

The National Council of Patient Information and Education, 815 Connecticut Ave., Washington, D.C. 20006, which includes 125 professional organizations, has been formed. It is hoped that this unsolicited help will not prove to be an intrusion into medical practice.

Contraception and compliance

It never ceases to amaze me that patients are willing to play "ovulation roulette" despite the availability of an arsenal of effective contraceptives, including the "morning after" pill. Perhaps they do not intend to become entangled in "amorous nets" so well described by John Milton. Long-acting contraceptives such as medroxyprogesterone acetate (Depo-Provera) seem to be a viable option for the poorly motivated. If women would insist that their partners use condoms, not only would contraception be served but sexually transmitted diseases could be prevented. I do not agree with Madame de Sévigné, who in 1671 called the condom an "armor against enjoyment and a spiderweb against danger."23

How can we achieve better patient compliance?

Good patient communication, individualized care of the patient, ease of medication use, and short courses of therapy are all helpful. The problem of patient compliance is of paramount importance and is vexing to all concerned. Improvement will require the combined efforts of physicians, nurses, pharmacists, the pharmaceutical industry, and, certainly not least, patients and their families.

Together we can accomplish much. To paraphrase John Donne, "Failure will diminish us all."

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Efficacy and tolerability of single-dose versus six-day treatment of candidal vulvovaginitis with vaginal tablets of clotrimazole

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One hundred ninety-nine female patients with candidal vulvovaginitis were included in an open, randomized, mycologically controlled study carried out at three Dutch gynecologic clinics to determine the efficacy and tolerability of a single vaginal tablet containing 500 mg clotrimazole in comparison with a 6-day treatment with vaginal tablets containing 100 mg clotrimazole. Both groups were comparable in age, body weight, and duration and severity of the infection, and in both groups the percentage of patients who were also treated for vulvitis with a 1% clotrimazole cream and the percentage of the male partners who were treated were equal. Four weeks after therapy, 84 of the 102 patients (82.4%) treated with one dose of 500 mg clotrimazole were cured and 82 of the 97 patients (84.5%) of the 6-day treatment group were cured. The clinical symptoms improved parallel to the improvement of mycologic findings. Both the vaginal tablets containing 500 mg and those containing 100 mg clotrimazole were well tolerated by the patients. After 1 week the results of the single-dose treatment with 500 mg clotrimazole were slightly better than those of the 6-day treatment with vaginal tablets of 100 mg clotrimazole. After 4 weeks the results were reversed, but it should be considered that this could be due to reinfection of the patients. (AM J OBSTET GYNECOL 1985;152:953.)

Key words: Candidal vulvovaginitis, clotrimazole, single-dose treatment

Yeast infections of the vulva and the vagina, especially those caused by *Candida albicans*, are very frequent. In fact, these infections are the most common cause of vaginal and vulval infections¹: yeast infections, 45%; *Gardnerella*-associated infections, 40%; *Trichomonas vaginalis* infections, 10%; leukorrhea of unknown cause, 50%.

In The Netherlands, most of these infections will be treated by the general practitioners. Nevertheless, in the gynecologic practices of the three gynecologists participating in this trial, many cases of yeast infections are seen, especially in women using oral contraceptives, in women who were recently treated with antibiotics, and in pregnant women.

The results of the treatment of yeast infections are remarkably improved since the introduction of the newer antimycotics, and one of the most effective treatments for *C. albicans* is clotrimazole.² The new trend in the treatment of yeast infections is the shortest possible therapy with the most effective dose of the active substance, because in longer-lasting therapy, many patients will stop treatment too soon.

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Patients fulfilling the following were included in the trial: (1) clinical evidence of vaginal and/or vulval yeast infection; (2) positive mycology [yeasts in the wet smear (pseudomycelia and/or buds) or a positive mycologic culture for *Candida* infection (Nickerson's medium)]; (3) informed consent of the patient; (4) willingness of the patient to return for follow-up control studies 1 and 4 weeks after the end of the treatment.

The main criterion was a positive mycologic culture at the start of the therapy.

Criteria for exclusion of the study were as follows: (1) pregnancy in the first 9 weeks, (2) trichomonal and/or bacterial vaginitis, and (3) concomitant oral and/or local antimycotic therapy.

Methods

In the three different clinics a total number of 214 patients with proven *Candida* vaginitis were treated with one tablet containing 500 mg clotrimazole or with six tablets each of which contained 100 mg clotrimazole and were administered during 6 consecutive days. The

Table I. Patient characteristics in the two study groups

		Clotri	mazole	
	1 × 5 (n =		5 × 10C mg (n = 5=)	
Characteristic	No.	%	No.	%
Status				
Primary	76	75	65	57
Recurrent	26	25	32	38
Severity				
Mild	61	60	54	5€ ·
Severe	41	40	43	14
Duration				
<1 wk	12	12	20	21
I wk to 1 mo	53	52	52	. 53
>1 mo	37	36	25	26
Hormonal contra- ception	13	13	14	14.
Pregnancy	20	20	20	21
Use of cream				٠,
Vulva	77	75	75	77
Glans penis	90	88	85	≋ 8
Sexual abstinence Local intolerance	96	94	88	9 1
Clotrimazole cream, 1%	3	3	3	3
Vaginal tablets	0		1	: 1

vaginal tablets were always inserted high in the vagina with a special plastic applicator. In the case of Condida vulvitis the patients were also treated during 6 days with a cream containing 1% clotrimazole, whicL vas applied once or twice daily in the vulval region Although the risk of a reinfection by the male pætrer seems to be low, this risk factor was excluded to the treatment of the male partners with the same cman, to be applied on the glans of the penis once or wice daily. Because of incomplete data, 15 patients had to be excluded, so that a total of 199 patients were evaluated. Of these, 102 patients were treated with one tablet of 500 mg clotrimazole and 97 patients were treated during 6 days with 6 tablets each of which contained 100 mg clotrimazole. Both investigated groups were comparable in mean age, mean weight, and mean height.

During the investigation, several data were noted:

- 1. Primary or recurrent vaginal candidiasis (In women successfully treated 6 months or longer the infection was classified as primary. In women who required treatment two or more times within the past year, the infection was considered to be recurren.)
 - 2. Severity of the infection: mild or severe
- 3. Duration of the infection: up to 1 week or Tom 1 week to 1 month or more than 1 month
 - 4. Method of contraception; existence of pregnancy
- 5. Concomitant medication, e.g., antidiabetics irramunosuppressants, corticosteroids, contraceptive or other hormones

- 6. Existence of a vulvitis and use of the 1% clotrimazole cream
- 7. Treatment of the male partner with a 1% clotrimazole cream and abstinence from sexual intercourse
- 8. Local tolerance of the clotrimazole vaginal tablets and/or the cream

For all these data the group using one tablet containing 500 mg clotrimazole corresponded relatively well with the group using six tablets containing 100 mg each, as shown in Table I. In the group using one 500 mg tablet there were a few more mild and primary infections, but the infections had already existed for a longer period of time.

Local tolerance. In connection with the high acidity of the 500 mg vaginal tablet (pH 3.8), local tolerance was recorded by questioning the patients. The local tolerance with the 500 mg vaginal tablet was excellent, and no side effects were reported that were related to application of these vaginal tablets.

The 1% clotrimazole cream, which was prescribed for all male partners and for women who had vulvitis, gave rise to irritation in six patients, divided equally between both groups of patients.

Clinical results

The clinical results were judged 1 and 4 weeks after the end of therapy by the persistence or absence of the following symptoms: itching, burning, vaginal discharge, vulvitis, and pathologic changes of the vaginal mucosa. All these symptoms were favorably influenced by both treatments.

The following remarks can be made:

- 1. Vulvitis was present in more than 70% of all patients and persisted slightly longer in the patients treated with one tablet containing 500 mg clotrimazole. However, in both groups, vulvitis was treated similarly with the 1% clotrimazole cream.
- 2. Itching and vaginal discharge were the main complaints. Both persisted slightly longer in the patients treated with one 500 mg tablet.
- 3. Burning was still present 1 week after treatment in a remarkably larger number of the patients treated with one 500 mg tablet, but 4 weeks after treatment the results in both groups were practically equal. Since treatment with six 100 mg tablets takes 5 days longer than that with one 500 mg tablet, the explanation could be that it takes about 2 weeks for this symptom to disappear.

Mycologic results

Follow-up was done only with those patients who, before treatment, had a positive wet smear and a positive culture on Nickerson's medium for *Candida*.

The results are shown in Table II. After 1 week the results in the group treated with one 500 mg tablet

Table II. Mycologic results

	Before treatment		After treatment							
				After 1 wk			After 4 wk			
			1 × 5	500 mg	6 × 1	100 mg	1 × .	500 mg	6 × .	100 mg
	1 × 500 mg	6 × 100 mg	No.	%	No.*	%	No.	%	No.	%
Wet film										
Negative	0	0	95		89		85		84	
Positive	102	97	7		7		17†		13†	
Nickerson's medium										
Negative	0	0	91	89.2	84	87.5	84	82.4	82	84.5
Positive	102	97	11	10.8	12	12.5	18†	17.6	15†	15.5

^{*}At that time, one patient could not be evaluated because of menstruation.

were a bit better than those in the group using six 100 mg tablets.

After 4 weeks the results in the group again using six 100 mg tablets were slightly better.

In the group treated with one 500 mg tablet, 18 patients had a positive culture on Nickerson's medium after 4 weeks. Of these patients, 17 had a subsequent treatment with the 100 mg tablets over 6 days; 4 weeks after this last treatment, 13 of these patients had a negative culture on Nickerson's medium.

Comment

For a comparison of the results of single-dose therapy with one vaginal tablet containing 500 mg clotrimazole and a 6-day treatment with vaginal tablets containing 100 mg clotrimazole each, the results of the mycologic examinations were considered to be the most important parameters.

One week after the treatment the results were slightly better with single-dose therapy than with the 6-day therapy: there were negative mycologic findings in 89.2% and 87.5%, respectively. Four weeks after the treatments the results were the reverse: a negative mycologic culture was obtained in 84.5% with the 6-day therapy and in 82.4% with single-dose therapy.

The difference in the results after 1 and 4 weeks might be due to a greater tendency toward relapses after single-dose therapy. In our opinion, however, it should be ascribed to a casual, more frequent occurrence of reinfection, especially since such a tendency was not observed in an earlier open study with singledose therapy.3

The overall conclusion should be that both treatments give favorable results. In connection with the problem of patient compliance, treatment with a single dose of 500 mg clotrimazole should in general be preferred to the 6-day treatment.

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[†]A number of 1-week failures were also considered as 4-week failures but could not be checked again because other antifungal therapy had been started.

Repeated candidiasis: Reinfection or recrudescence? A review

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Candida vulvovaginitis is one of the most common infec∃ors encountered by obstetricians and gynecologists from all parts of the world. The modern treatment of vaginal candidiasis with imidazcle antimycotics such as clotrimazole has been shown to be nightly effective. However, there is a small group of women who experience repeated episodes of vaginal andidiasis. This article reviews possible predisposing factors related to recurrent vaginal candidiasis (AM J OBSTET GYNECOL 1985;152:956.)

Key words: Vaginal candidiasis, predisposing factors

The yeast Candida albicans is frequently found in the vagina. Approximately 30% of pregnant and 15% of nonpregnant women harbor Candida in the vagin_1 In 1981, Fleury² reported on a recent prospective study in which 20.5% of 20,000 unselected patients harbored C. albicans. Approximately half of these carriers have or will develop candidal vulvovaginitis. The concetion gives rise to intense burning or itching and væir al discharge. Imidazole antimycotics such as clotrim=zcle have been shown to be highly effective in the treatment of candidal vulvovaginitis.3.5 However, there is a nall group of women who experience repeated episoæs of candidal vulvovaginitis.⁶ In this group of women it is often difficult to decide whether the next episode represents a reinfection or a recrudescence of the pre-ious episode, during which the organism was severel-cepleted but not completely eliminated.

When women with repeated episodes of candidavilvovaginitis are treated, it is of utmost importance to consider possible predisposing factors for candidal infection. These predisposing factors can be divided into three main groups:

- 1. Fungal inoculation
- 2. Nutrient availability
- 3. Defense system disorders

Fungal inoculation

Gastrointestinal reservoir. The gut frequently contains asymptomatic Candida, and it has been suggested that this fecal reservoir may well represent a source of infection in cases where an apparently successful radication of the organism from the vagina is subsequently followed by another attack. The Recent studies of normal adults show that C. albicans can be recovered from the oropharynx in 30%, the jejunum in 50%, and the Eeum in 50%, as well as from 65% of normal fecal sameles.

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Using the resistogram method of strain differentiation, Warnock et al.¹¹ have demonstrated that most patients with genital candidiasis also had the same strain of *C. albicans* in the digestive tract. In cases of recurrent vaginal candidiasis, treatment with oral nonabsorbable polyene antibiotics such as nystatin has been evaluated.¹² Local treatment with clotrimazole was compared with clotrimazole given locally plus oral treatment with nystatin. The cure rates and relapse figures were similar in both groups. If oral treatment is given, intestinal recolonization occurs once treatment is discontinued. A new report recommended use of clotrimazole cream around the perianal region.¹³ It is possible that the frequent use of these creams may prevent recurrences.

Sexual contact. Sexual contact is probably a contributory but not highly significant factor. If it were important in spreading the disease, then we might reasonably expect a significant number of men to carry the fungus on the penis. However, several studies have failed to confirm this expectation. ^{14,15} In the most recent of these studies, ¹⁵ C. albicans was isolated from the penis of only 5% of new patients attending a venereal diseases clinic. Several studies have failed to report improvement of results when partner treatment is given. ^{16,17}

Nutrient availability

Pregnancy. Numerous reports have stressed the fact that *C. albicans* is more commonly harbored in the vagina of pregnant patients than in that of her nonpregnant counterpart.^{1, 18} Although the reported incidences vary greatly, approximately 30% to 35% of pregnant women have positive vaginal cultures for *Candida* in comparison with 15% of nonpregnant women. The high levels of estrogen during pregnancy result in an abundance of glycogen in the vaginal mucosa, providing an ample supply of utilizable sugars and thus favoring fungal growth. The incidence of vaginal yeast infections in pregnant women continually increases throughout pregnancy to the time of delivery. Follow-

ing delivery, the regression of the vaginal epithelium and the disappearance of glycogen removes the factors enhancing fungal growth. These changes are reflected in a rapid disappearance of Candida from the postpartum vagina.

Diabetes mellitus. Diabetes mellitus is usually considered to be one of the conditions that predispose to infection by Candida. 1, 18 Studies based on culture results clearly prove an increased incidence of Candida colonization in diabetics. The incidence of candidal vulvovaginitis is increased among women with poorly controlled diabetes mellitus, in whom high serum glucose levels are frequently recorded.

Regular intake of a carbohydrate-rich diet has also been incriminated in the etiology of recurrent candidal vaginitis.19 It has been suggested that this factor bears resemblance to diabetes mellitus by offering higher tissue glycogen levels and even increased fungal multiplication in the gut. In clinical work this factor seems to have received little attention.

Oral contraceptives and intrauterine devices. Several studies have indicated a statistically significant increased incidence of candidal vulvovaginitis among women using oral contraceptives.20-22 The incidence of vaginal mycoses among women using oral contraceptives was evaluated by Wied et al.20 in 1966. The ratio of control patients with candidiasis who were not using oral contraceptives to patients with candidiasis using the pill was 4.5% to 8.5%. This represents an increase in candidiasis of nearly 80% among women using oral contraceptives. The use of oral contraceptives results in an increased availability of vaginal glycogen, thus favoring fungal growth. In women using oral contraceptives the glucose content of the vagina is increased by 50% to 80% in comparison with untreated control subjects. The pH of the vaginal contents rises to as much as 6.5 in women using hormonal contraception.25 The duration and type of oral contraception used have, however, been reported to influence the incidence of symptomatic infections. The frequency of candidal infections does not appear to be affected by modern contraceptive tablets containing very low doses of steroids. DeCosta²⁴ suggested that the increase in candidiasis in patients using oral contraceptives is based on nonspecific factors rather than endocrinologically mediated mechanisms. Such factors in patients using birth control pills might include an increased frequency of intercourse, the failure to use a condom (thus promoting spread of infection from male to female partner), and the failure to use contraceptive creams that may be fungistatic. However, it has been reported that the incidence of candidal vulvovaginitis increased in women taking estrogens for climacteric symptoms. Thus, although the facts mentioned by DeCosta24 may be of importance, endocrinologic mechanisms are certainly also involved.

The incidence of vaginal candidiasis is reported to be increased among users of an intrauterine device.25 This high rate of morbidity among these women may be attributable to local changes in resistance because of increased secretion resulting from foreign body contamination and the promotion of fungal colonization.

Antibiotics. Antibiotic therapy has clearly been shown to result in an increased incidence of candidal vulvovaginitis. 1, 25, 26 The most likely explanation for this fact appears to be the suppressive effect of antibiotic agents on susceptible bacteria, thus permitting overgrowth of resistant organisms, including Candida. Whether suppression of the bacterial flora permits candidal overgrowth by eliminating bacteria that actively compete for nutrients or by eradicating bacteria that inhibit fungal growth is unknown. The present consensus is that the former is probably the most important single factor. Antibiotic-induced candidal infections seem to be particularly frequent following systemic administration of broad-spectrum antibiotics. It has also been reported that antibiotics, particularly tetracyclines, have a direct growth-stimulating effect on fungal colonization.²⁷ Antibiotic therapy may also promote the intestinal proliferation of yeasts by destruction of the normal fecal flora. The enhancement of this fecal fungal reservoir may in turn influence vaginal colonization with yeasts.

Defense system disorders

Host-organism relationship. The question of why certain individuals are susceptible or even repeatedly susceptible to candidiasis, whereas others remain free from symptomatic illness even though they may be carrying the organism as a saprophyte, has puzzled many specialists in the field of mycology. What is the mechanism that triggers the onset of symptomatic disease? It seems likely that the change occurs within the host itself rather than the fungus. At present there is no adequate explanation for this transition from an asymptomatic condition to symptomatic illness.

Immunologic disorders. Certain families with a genetically determined defect in cellular immunity or patients with iatrogenic drug impairment of normal cellular immunity have an increased susceptibility for both systemic and local candidal infections.1

The immunologic basis of recurrent candidal vaginitis has been extensively studied by Hobbs et al.28 Humoral immunity does not appear to play a major role in the resistance to C. albicans because most women possess antibodies to this organism. Hobbs et al. also recorded the fact that women with defects in the sequence of events leading to antibody formation did not have an increased incidence of *C. albicans*. However, the same authors demonstrated a 65% incidence of subnormal or borderline in vitro Y-lymphocyte responses to candidal extract.

Witkin et al. 29 investigated the ability of *C. albican*, to inhibit lymphocyte proliferation in women with ecurrent vaginal candidiasis. Proliferation of peripheral blood lymphocytes was clearly inhibited in the presence of *C. albicans* extract. Furthermore, the lymphocytes from patients with recurrent candidal vaginitie suppressed the proliferative response of control lymphocytes to *C. albicans*. The authors concluded that women with recurrent *C. albicans* vaginitis appear to produce *Candida*-specific suppressor lymphocytes, which plack the cellular immune response to this organism.

Adrenal steroids. Administration of adrenal elucocorticoids clearly enhances experimental infertions caused by a variety of microbial agents. Recent studies suggest that corticosteroids may predispose to hot cell destruction by stabilizing the lysosomal membrane thus preventing the release of catabolic enzymes that prelinarily digest phagocytized organisms. Although the administration of adrenal steroids clearly predisposes to systemic candidiasis, there is at present little information regarding the association between steroids and the development of vulvovaginal candidiasis.

Stocking tights and jeans. The wearing of sto king tights or tight jeans is believed to severely lime the circulation of air around the vulva. The effect is thus to produce a warm, moist environment that is ideal for the growth of *C. albicans*. Local occlusion and maceration predisposes to candidal overgrowth and infection. There are reasons to believe that the wearing of socking tights or jeans predisposes to genital candidal infection by this mechanism. ^{26, 30} Hydrolytic enzymes on the surface of *C. albicans* cells contribute to the damage of host cells by the fungus. The *Candida* organism. The candida organism.

Comment

The possibility of predisposing conditions should always be considered in cases of recurrent vaginal candidiasis. Reduction or elimination of possible predisposing factors is an integral part of the treatment of recurrent candidal vulvovaginitis. Further research is, however, necessary to improve our understanding of the possible factors leading to recurrent genital candidiasis. The development of techniques to differentiate strains of *C. albicans*³¹⁻³⁸ provides a useful too for future research. With such techniques it may be possible to determine whether or not repeated cancidal infections represent a reinfection or a recrudescence

of the previous episode. It is possible that these techniques will improve our understanding of predisposing factors such as sexual contact and the gastrointestinal reservoir.

The recognition of the importance of cellular immunity in the pathogenesis of recurrent vaginal candidiasis is an exciting new development. The ability of women with recurrent genital candidiasis to produce *Candida*-specific suppressor lymphocytes is of great interest and warrants further research.

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Treatment of recurrent vaginal candidiasis

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In recurrent vulvovaginal candidiasis, predisposing factors should be eliminated wherever possible. Reduction of the gastrointestinal yeast flora by oral antimycotic treatment does not prevent recurrences. Perianal application of antifungal cream might be an alternative in preventing reinfection from the rectum. Venereal spread of yeasts does not seem to be an important factor, and partner treatment does not significantly alter treatment results. Traditionally, prolonged treatment periods are recommended to prevent recurrences. Patient compliance may then become a factor limiting efficacy. One-dose treatment has been shown to give results that compare favorably with traditional treatment schedules. Results in recurrent cases were comparable with those in primary infections. Thus one-dose treatment could be an acceptable alternative to longer treatment periods in schedules for treatment of recurrent cases. Intermittent prophylactic one-dose application appears to be a promising method of reducing recurrences. (AM J OBSTET GYNECOL 1985;152:959.)

Key words: Vulvovaginal candidiasis, prophylactic treatment

The group of patients suffering repeated vulvovaginal candidal infections presents a challenge to the gynecologist. Numerous schedules for the management of these cases have been designed. However, most of us have experience with the virtually intractable pa-

In the search for predisposing factors, the patient should be screened for diabetes or for use of antibiotics. In most cases, however, no such factor is found, or when found, it is impossible to eliminate.

Attempts have been made to reduce the gastrointestinal reservoir to prevent reinoculation of the genital region. Oral treatment with polyene antibiotics, e.g., nystatin or amphotericin B, is often prescribed, but results seem disappointing. For instance, in a doubleblind study, local treatment with clotrimazole was com-

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pared with clotrimazole used locally in combination with oral treatment with nystatin. The cure rates and relapse figures were similar in both groups. If oral treatment is given, intestinal recolonization often occurs once the treatment is discontinued. Using the resistogram method for strain differentiation, Warnock et al.2 demonstrated that most patients with genital candidiasis also had the same strain of Candida albicans in the digestive tract. Oral treatment did not eradicate the yeasts from the gastrointestinal tract. Another method of reducing the spread from the rectum to the vulva and vagina has been to apply antifungal cream to the perianal region and perineum.3 Such prophylactic treatment has been shown to reduce significantly the recolonization of the vagina.

Treatment of the male partner to reduce the risk of sexual reinfection is also recommended. Even if venereal spread cannot be excluded as a factor predisposing to recurrences, it does not appear to be an im-

Table I. Results at 4 weeks in primary cases

	Dosage schedule			
	500 mg,	200 mg,	100 r.g,	
	single dose	3 days	4 or 6 days	
	(n = 33)	(n = 37)	(n = -2)	
Clinically cured	31	34	51	
Colonized	4	3	5	

portant factor, since several studies failed to report improvement of results when partner treatmen was given.4.5

Hygienic methods such as restricting the use of scap and water for vulval cleansing and the use of ottn underwear rather than nylon have been recommended. Underwear should be washed at temperatures exceeding 80° C, and close-fitting tights or jeans should be avoided, according to Hurley.

In a literature survey, Odds⁷ concluded that reapse rates were highest in patients who were treated for the shortest time and lowest in patients treated for the orgest time. In addition, prolonged treatment periods are often recommended for patients with frequent recurrences.

Since symptomatic relief is usually obtained a et a very short period of treatment, patient compliance becomes a problem when prolonged treatment is prescribed. In a study of patient compliance, Mast room et al.8 found that 4% of patients never started to take their medication, and half interrupted treatment explier than prescribed.

Intermittent prophylactic treatment using loca application of clotrimazole for 6 days during each neastrual cycle has been shown to prevent symptomatic recurrences. Recolonization of the vagina, however, was not prevented with this type of treatment.

Since it has been shown that one-dose treatmentwin modern imidazoles gives results that are comparalle to those with older treatments, it was considered of interest to see whether there were any difference between the results in groups having recurrent and shose having primary cases of vulvovaginal candidiasi. At the World Congress of Gynecology and Obstetres in San Francisco in 1982, we presented a study comparing three different dosage schedules.10 Traditional Eday treatment using 100 mg clotrimazole daily, a 5-day treatment using 200 mg clotrimazole daily, and one single dose of 500 mg clotrimazole administered by pessary were compared. The results in the three testment groups did not differ significantly. In xoxtrol studies 4 weeks after treatment, rates of clinical cure (defined as absence of symptoms and yeas net identifiable in the wet smear) were 82% in the €-day group, 92% in the 3-day group, and 94% in the 1-

Table II. Results at 4 weeks: Recurrent episodes

	Dosage schedule				
	500 mg $(n = 18)$	$200 \text{ mg} \ (n = 19)$	$ \begin{array}{c} 100 \text{ mg} \\ (n = 20) \end{array} $		
Clinically cured Colonized	16 2	16 0	17 1		

day group. Mycologic cure rates were 74%, 84%, and 82%, respectively. The differences are not statistically significant, and the results are in the same range that is usually reported in vulvovaginal candidiasis (Table I).

According to the traditional view, however, one would expect that recurrent cases might show lower cure rates. No such tendency was observed when all recurrent cases were compared with all primary cases. There were 75 primary cases with a clinical cure rate of 89% and a mycologic cure rate of 77%. There were 57 recurrent cases with a clinical cure rate of 86% and a mycologic cure rate of 81%. Recurrent cases were also grouped according to type of treatment. These groups were small, and the results in Table II have to be evaluated cautiously in view of that fact.

One-day treatment seems to be an effective means of dealing with the acute episode of vulvovaginal candidiasis even in recurrent cases. The need for patient compliance is minimal, since only one local application is necessary.

No single effective therapy can be recommended in recurrent vulvovaginal candidiasis. For the acute situation, single-dose treatment with 500 mg clotrimazole is an alternative to traditional treatment schedules. For prevention of repeated episodes, prophylactic intermittent treatment using a single dose of 500 mg clotrimazole appears to ensure maximum patient compliance. Oral treatment with antimycotics seems of limited value, but perianal application of an antimycotic could be an effective alternative preventing recolonization from the rectum. Partner treatment is of doubtful value in attempting to improve treatment results in the female patient. The major breakthrough in the treatment of recurrent cases has yet to come; it seems reasonable to expect it to be in the field of immunology.

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Is more than one application of an antifungal necessary in the treatment of acute vaginal candidiasis?

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The results of all the controlled trials carried out at the Department of Genito Urinary Medicine at the Cardiff Royal Infirmary over the past 16 years are summarized. All except ore of these trials were carried out with patients having acute vulvovaginal candidiasis. One trial involved treating only patients with recurring candical infection. In all the acute trials, there were practically no mycologic relapses 7 days after completion of treatment whatever the regimen used, but at 35 days after completion of treatment the mycologic relapse rate was in the region of 20% to 25%. It is concluded that following the elimination of any known precisposing cause of vaginal candidiasis, the intravaginal application of 500 mg of an imidazole preparation is as effective a treatment as any other regimen. In recurrent cases, monthly treatment with such a dose may be adequate to control the patient's symptoms. Mycologic relapse may not be accompanied by symptoms, but in recurrent cases there is a closer relation between mycologic relapse and symptoms. (Am J OBSTET GYNECOL 1985;152:961.)

Key words: Acute vaginal candidiasis, nystatin, tioconazole, econazole, clotrimazole

Ever since the introduction of effective antifungal agents, various regimens of treatment with local pessaries, tablets, creams, tampons, and even foaming pessaries have been tried. These regimens have varied from 14-day courses of nystatin,' to 15-day courses of miconazole-impregnated tampons,2 to a 3-day course of clotrimazole tablets, 200 mg daily.3 The cure rates in these trials have varied from an alleged 97.4%2 1 month after treatment to 89.4%.3 Masterton (personal communication, 1979) has noted, as have others, that the first attacks of vaginal candidiasis respond better to treatment than do those in patients with a history of previous infections. However, he found that with longer follow-up, the relapse rate rose. Several attempts have been made to assess the relationship between anorectal-colonic candidiasis and vaginal candidiasis, 1, 4, 5 but combined oral and vaginal therapy has proved to be of no more value in the long term than local therapy.

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Many statements have been made on the basis of these investigations over the years.

Odds,⁶ in 1977, stated that the cure rate of vaginal candidasis after 6 to 17 days of treatment was 94%, whereas the cure rate if the treatment lasted less than 6 days was only 47%. In 1981 Odds and MacDonald⁷ were stating that the overall cure rate using the imidazole group of drugs was 90%. However, the overall cure rate using the polyene group of drugs was only 79%.

In 1974 Cho et al.⁸ said that "vaginal pessaries alone are less effective in the treatment of vaginal candidiasis than in a combination of pessaries and ointments." By 1976 Masterton et al.⁹ were saying that of the patients who were seen for vaginal candidiasis, 4% did not start treatment and 50% did not finish treatment. More importantly, in 1981 Odds and MacDonald⁷ reported that in the treatment of vaginal candidiasis the total dose may be more relevant than the duration of treatment.

In relation to recurrent candidiasis, many statements of importance have been made over the years. In

1975 Hurley¹⁰ maintained that in recurrent candidiasis, nystatin pessaries should be used twice day for 6 weeks, in addition to local application of eam. Masterton et al.³ were saying in 1977 that there is no place for short courses of therapy, even though Masterton (personal communication) had initially advected shorter and shorter therapies of treatment. Davidson and Mould¹¹ in 1974 recommended that clotricatole should be prescribed as a 100 mg pessary twice daily from the fifth to the eleventh day of the menstrual cycle in women with recurrent vaginal candidiasis.

In order to put all these statements to the est, I decided to publish (1) the results of five trials carried out with the use of an antifungal in patients with acute candidiasis and (2) the results of one trial (trial 6 in recurring candidiasis.

Patients and methods

Randomized trials and balanced control studies were conducted in a static population of about 300,00% in a small principality town in Cardiff, Wales. We have good patients in that they come back for the trials and are somewhat of a captive audience. Over the years, the trials have involved the polyene group of drugs and, more recently, the imidazole group.

In all the trials, women over the age of 16 year who came to a clinic of genitourinary medicine with plastical signs of acute vaginal candidiasis were entered, with their verbal permission, into randomized trials. So that the compliance with the criteria of all the trials vould be obtained, patients were selected who came for the first time in 3 months with an attack, who had a nigh vaginal swab that was positive for Candida albicane and who had no concomitant or mixed genital infection with Neisseria gonorrhoeae, Trichomonas vaginalis, herperirus hominis (herpes genitalis), or Haemophilus vaginala Patients were excluded from any trial if they were uncer the age of 16 years, if they did not give verbal coasent to enter the trial, or if they had been treated with any vaginal topical agent in the previous 4 weeks or were known to have any sensitivity or an allergic reaction in the past to any of the imidazole or polyene groups of drugs. They were not entered into the trial if they were thought to be mentally ill or unlikely to cooperate w th the requirements of the study or if they were menstruating at the time of initial examination. Premant women were also excluded.

Patients who had been assigned to the study on the basis of an initial clinical diagnosis but who were subsequently found to have had negative mycologic cultures were excluded from the main analysis. However, provided they returned for the first follow-up ve.t at day 7, their data could be included in the overall-subjective analysis on acceptability and improvement of

symptoms. Any patients who did not return for the day 7 follow-up would be excluded from the analysis.

Clinical diagnosis was confirmed by mycologic diagnosis. Two swabs were taken from the posterior fornix. One dry swab was plated onto Sabouraud medium, incubated for 48 hours at 37° C, and then examined for candidal colonies. The second swab was placed in *Trichomonas* culture medium supplied by Medical Wire and Equipment Co. (Ref. No. MW 220). The second swab was examined for colonies of *Candida* at once and after 48 hours of incubation at 37° C. If yeasts were found on the wet preparation, this was plated onto Sabouraud medium for 48 hours at 37° C.

Colonies of *Candida* were incubated in plasma at room temperature and examined microscopically for germ tubes. If germ tubes were present, the colonies were incubated at room temperature in corn meal agar and examined for chlamydospores daily for up to 5 days.

Patients fulfilling the criteria for admission to any trial were examined by the investigator and allocated a trial number. At this stage, the following were recorded: (1) stage of menstrual cycle; (2) previous antimycotic therapy in the past year; (3) date of any previous attacks of vaginal candidiasis; (4) possible predisposing factors, including accompanying disease and drugs, diabetes, immunodeficiency diseases, iron or zinc deficiency, oral contraceptive use or oral steroid use, and recent use of antibiotics; (5) duration of present infection; (6) severity of symptoms of discharge, irritation, and burning according to a four-point scale.

The investigations included broad screening for immunosuppression so that the subjects would have a normal differential white blood cell count. They all had a fasting blood glucose test; however, since the pickup rate of diabetes is almost nil, the test is a waste of time. Assays of serum levels of iron, folic acid, and vitamin B_{12} were also done; at the end of 16 years, I believe that they also are a waste of time. A complete urinalysis, which is not a waste of time, was also done. The following trials involving patients with acute vaginal candidiasis were carried out:

- Nystatin pessaries: two at night for 15 nights involving 50 patients, compared with amphotericin
 B pessaries, one at night for 15 days involving 50 patients. The patients were randomly selected.
- 2. Nystatin pessaries: one at night for 6 nights. One hundred consecutive patients were used in this trial.
- 3. Nystatin pessaries: two at night for 15 nights, plus oral nystatin four times a day for 10 days. Fifty patients were placed in this group and were compared with a second group of 50 patients who were given amphotericin B pessaries, one at night

for 15 nights, in combination with oral nystatin tablets, four times a day for 10 days. Again, there was random selection of patients.

- 4. In 50 patients a 3-day course of tioconazole cream was prescribed; these patients were randomly compared with 50 patients who received one pessary a day containing econazole.
- 5. In the final trial involving patients with acute vaginal candidiasis, either a single pessary containing 500 mg clotrimazole or two pessaries containing 300 mg isoconazole were inserted into the vagina by the investigator, who used an appropriate applicator according to random selection. Clotrimazole cream was given to the patient for treatment of the sexual partner, and in all trials the patients were asked to refrain from sexual intercourse until after the first revisit.

Follow-up examination in all trials was carried out at day 7 and at day 35, at which time two mycologic specimens were taken and the relevant physical signs and symptoms recorded. Patients were asked at day 7 to given their opinion on the acceptability of the preparation administered. Patients found to have a positive culture for Candida at day 7 were regarded as having . had treatment failures. Adverse reactions were noted.

In the trial involving 19 patients with chronic relapsing candidiasis (trial 6), the patients were chosen because they had had at least two single episodes of recurring candidiasis in the 3 months before entering the trial. Each patient in this prolonged trial was given a pessary containing 500 mg clotrimazole at weekly intervals and was randomly selected to receive either four or five pessaries. Only 19 patients were entered into and completed this trial. They were then examined mycologically 2 weeks after completion of treatment and then at the end of 1, 2, 3, 6, 9, and 12 months.

Results

The overall mycologic cure rates for patients enrolled in the five trials involving acute candidiasis and for patients enrolled in the trial involving recurrent candidiasis (trial 6) are described below.

Trial 1. In the comparison of nystatin pessaries (two at night for 15 days) and amphotericin B (one at night for 15 days), there was a failure of treatment in one patient in the nystatin group. Thirty-five days after completion of treatment, relapse occurred in 25% of patients in the nystatin group and in 24% of patients in the amphotericin B group.

Trial 2. Of the 100 patients treated with one nystatin pessary at night for 6 nights, there were four failures 7 days after treatment and a relapse rate of 29% 35 days after treatment.

Trial 3. In the series in which a regimen of two ny-

statin pessaries at night for 15 nights in combination with oral nystatin, four times a day for 10 days, was compared randomly with a regimen of one amphotericin B pessary at night for 15 nights in combination with oral nystatin, four times a day for 10 days, the failure rate in the amphotericin B group was 2 of 50. However, at day 35 after completion of treatment, 24% of the patients taking the nystatin combination had had a relapse and 21% of the patients taking the amphotericin B combination had had a relapse.

All the trials had almost exactly the same results. In fact, in 39% of patients who had rectal examinations in confunction with possible vaginal-associated infections of the rectum, Candida was isolated from the anal canal with a proctoscope before the trial. On the thirtyfifth day after treatment, Candida was isolated from 38% of the patients, indicating that treatment was a waste of money.

Trial 4. One of the more recent trials was an open trial comparing tioconazole with econazole. Seven days after treatment there were no relapses in the tioconazole group, but 2 of 50 patients were mycologically positive in the econazole group; i.e., the treatment failed in two patients in this latter group. Thirty-five days after treatment there was a 25% relapse rate in the tioconazole group, exactly the same as in the econazole group.

Trial 5. In the double-blind study comparing a 500 mg clotrimazole tablet with a single dose of two 300 mg isoconazole tablets, the overall mycologic cure rate was 74% with clotrimazole and 78% with isoconazole. There were no mycologic failures with clotrimazole. Only 45 of the original 50 patients using isoconazole returned at the end of the 35 days after treatment. In those 45 patients, there was a 22% failure rate, with patients showing signs and symptoms of candidal infection.

Trial 6. An extended single-dose clotrimazole regimen was attempted in the treatment of chronic vaginal candiciasis, defined as more than two attacks of proven, untreated vaginal candidiasis in the previous 3 months. The women in this trial were to return on a regular basis over 1 year and would be eliminated from the trial if they developed other infections. This protocol meant ending up with a relatively small study population. The results, however, do tell us something.

Of 19 patients, eight remained mycologically clear and asymptomatic for the whole year after their initial treatment. The remaining 11 patients became mycologically positive at varying intervals during the succeeding year after treatment. It was noted by the observers that there was a remarkable correlation in this group between mycologic positivity and the onset of symptoms. However, these patients were given no further treatment during the course of the year's followup. All these patients had a spontaneous remission, some again having a relapse, so that at the end of 1 year after treatment, six patients were again symptomatic and mycologically positive. They were removed from the trial and given a single dose of 500 mg clotrimazole.

This experience suggests that perhaps it is not necessary to treat so dramatically the patients in whom candidal spores are found, but certainly patient must be treated for their symptoms.

Summary of trials. In all the trials there was an extremely low default rate. Our patients were extramely well motivated, and a great deal of time and patience were spent in discussing the trials with the patients before admission.

There were no sensitivity reactions to the property and group of drugs. Two patients receiving clotric asole and one receiving isoconazole were excluded at tay 35 because they had started additional antibiotic therapy. One patient with side effects from the isoconazole was withdrawn because of these side effects. Two prients being treated with isoconazole developed severe side effects consisting of edema and redness of the vulva and vagina, and one was taken off the trial and treated by painting of the affected area with 1% aqueous solution of gentian violet. One other patient given conazole complained of slight irritation and burning, as did two patients who had been given clotrimezole.

Comment

Overall, the failure and relapse rates in all therials over the past 16 years involving patients with acute vaginal candidiasis have been found to be statistically similar.

In our series there has been no difference, owe the many years that we have conducted these trials, at the eventual mycologic outcome 35 days after competion of treatment. The recurrence rate in our series sing a single pessary with 500 mg clotrimazole was slightly higher than that found by other workers. Improvement in clinical signs correspond to mycologic enters. This correspondence does not relate to the findings of Davidson and Mould in 1974, who found dissoction between symptoms and the presence of vaginal reasts when they were studying recurrent genital candinasis in women and the effect of intermittent prophylactic treatment. In our recurrent series, we found, however, that there was a close relationship between mycologic

positivity and symptoms in the patients. All regimens that we used were well tolerated by all the patients entered into our trial, although two patients being treated with isoconazole were found to have severe local erythema and edema, with the result that one patient was removed from the trial. These two were the only patients in the whole of our series who found their treatment unacceptable.

Although the cure rates at 35 days after treatment were not as high as one would have wished, single-dose regimens are an important development in the treatment of vaginal candidiasis because they encourage patient compliance. If the investigator actually gives the treatment, 100% patient compliance is ensured. I also believe that there is a place for single-dose treatment in cases of recurring vaginal candidiasis in which no provoking cause can be found and in which no other regimen has been found to provide a successful, permanent cure of the condition. This regimen could be administered in a single-dose treatment by the patient at any time that her symptoms and signs recurred, provided, of course, that any other genital infecting agents were absent.

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Efficacy of single- versus multiple-dose clotrimazole therapy in the management of vulvovaginal candidiasis

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Los Angeles, California, and Metairie, Louisiana

Single-dose treatment of vulvovaginal candidiasis with a 500 mg clotrimazole vaginal tablet was compared to 3-day treatment with two 100 mg vaginal tablets administered daily in 115 patients enrolled in a double-blind trial, 101 of whom were evaluated for efficacy. Patients with clinically and mycologically active disease were treated (visit 1) and examined at 5 to 10 days (visit 2) and again at least 27 days (visit 3) post treatment. At visit 2, mycologic tests and clinical examinations were negative in 37 of 48 patients receiving single-dose treatment (77%) and in 47 of 53 patients receiving 3-day treatment (89%). Corresponding results for visit 3 were 65% and 74%, respectively. There were no significant differences in treatment response between groups, and only three patients reported adverse reactions. These data show that single-dose treatment with clotrimazole, 500 mg, is as safe and effective as the more complex 3-day regimen. (AM J OBSTET GYNECOL 1985;152:965.)

Key words: Vulvovaginal candidiasis, clotrimazole therapy

Clotrimazole is a synthetic antifungal agent used in the treatment of vulvovaginal candidiasis. The mechanism of action of clotrimazole is believed to involve the inhibition of fungal sterol synthesis, specifically the inhibition of formation of ergosterol—an essential constituent of the fungal cell membrane. The degree of inhibition of ergosterol biosynthesis by clotrimazole is a function of drug concentration. At low concentrations, only partial inhibition occurs, resulting in a fungistatic effect. At higher concentrations, clotrimazole may completely block ergosterol synthesis, producing a fungicidal effect.

The antimycotic properties of clotrimazole facilitated the shortening of treatment for vaginal mycoses from approximately 14 days to 6 or 7 days when a 100 mg vaginal tablet or 5 ml of a 1% vaginal cream was used. Increasing the dose was found to reduce the required treatment period to only 3 days. This study is designed to see the effect of a 500 mg vaginal suppository.

Efforts to shorten the treatment period even further by developing a more concentrated form of the drug have been complicated by the relatively low water solubility of clotrimazole. Water solubility of the drug at pH values of less than 4 was known to be four times greater than its solubility at a neutral pH. Bioavailability studies showed acid formulations of clotrimazole to be associated with an increased rate of drug release. Vaginal secretion concentrations of clotrimazole in patients using one 500 mg suppository were found to produce a zone of inhibition at 24, 48, and 72 hours consistently greater than that produced for secretions in patients

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using 100 or 200 mg applications nightly for the same period. Consequently, lactic acid-sodium lactate was used for acidification of the clotrimazole formulation, and a 500 mg vaginal tablet was developed.

Quantitative subculture tests showed this new formulation to have a killing rate of greater than 99.19% for *Candida albicans*.

For determination of clinical efficacy of once-daily administration of the 500 mg lactic-acid formulation of clotrimazole, a double-blind multicenter study comparing this regimen was conducted in patients with vulvovaginal candidiasis.

Material and methods

Women 18 years of age and older with clinically diagnosed and mycologically confirmed vulvovaginal candidiasis of recent onset were considered for enrollment in this study. The clinical diagnosis was based on the presence of six signs and symptoms: vulval irritation, vaginal irritation, discharge, pruritis, burning, and dyspareunia. These disease manifestations were graded on a scale of zero to three. A total score of at least three was required for entry into the study. Microscopic examination (potassium hydroxide) and cultures were used for mycologic confirmation of vulvovaginal candidiasis.

Patients having continued treatment with tetracycline or erythromycin were excluded. Women who used contraceptive foams, creams, or jellies were not eligible, although the use of an oral contraceptive drug, intrauterine device, or diaphragm alone was permitted. Patients in whom early pregnancy was suspected and patients pregnant less than 13 weeks were excluded from the trial. Other exclusion factors included hypersensitivity to the imidazoles; concomitant vaginal infection .

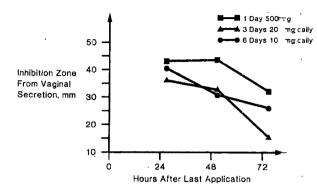


Fig. 1. Inhibition zones of vaginal secretions.

with Trichomonas, Gardnerella vaginalis, or group B streptococcus; a history of diabetes mellitus; and treament with exogenous estrogens.

Written informed consent was obtained fro □ all study participants, at which point patients were rendomly assigned to one of two treatment groups.

Group 1 received one vaginal tablet containing 550 mg of clotrimazole with appropriate placebo; the ther group received two 100 mg clotrimazole vaginal tablets daily for 3 days.

At the initial visit a physical examination was conducted and vaginal specimens were obtained for microscopic evaluation and culture. The investigator ham placed three tablets deep into the vagina. Women in group I received one 500 mg clotrimazole table and two placebo tablets. Women assigned to group \geq had two 100 mg clotrimazole tablets and one placebo \rightleftharpoons bet inserted into the vagina. Both groups then inserted two tablets into the vagina on days 2 and 3; in group \perp the tablets were placebo, and in group 2 they were active drug, 100 mg.

Patients were instructed to return 5 to 10 days later for visit 2 and again after 27 to 31 days for visit 3. At these visits the signs and symptoms of cancidiasis sere again graded and vaginal specimens were obtaine I for microscopic examination and culture. Adverse exactions were also recorded at each visit.

At the final visit the overall outcome of treatment was evaluated. Successful treatment was defined as the absence of signs and symptoms of vulvovaginal candidiasis, coupled with negative mycologic findings. The persistence of signs and symptoms or positive mycologic findings constituted treatment failure.

Fisher's exact test for categorical data and Studen's t test for continuous data were used for analysis of the baseline demographic and clinical characteristics of the two treatment groups. Pretreatment comparability of signs and symptoms of vulvovaginal candidiasis waldetermined by using a nonparametric Wilcoxon's fark test. Fisher's exact test was used to compare treatment efficacy.

Table I. Baseline patient characteristics of patients evaluable for efficacy

	Clotrin	nazole
	500 mg $(n = 48)$	$ \begin{array}{c} 200 \text{ mg} \\ (n = 53) \end{array} $
Age (yr)		
Mean	29.1	29.2
Range	19.0-71.0	19.0-59.0
Weight (lb)		
Mean	132.3	138.8
Range	104.0-186.0	99.0-212.0
Duration of disease (mo)		
0-1	41	51
>1, <6	7	2
Pregnancy status		
Pregnant (>13 wk)	44	48
Nonpregnant	¹ 3	3
No information	1	2
Contraceptive use		
Oral contraceptive	20	20
Intrauterine device	1	0
Other	10	11
None	17	21
No information	0	1
Recent medication	•	
Yes	2	2
No	45	50
No information	1	1
Concomitant therapy		
Yes	0	2
No	48	51
Signs and symptoms		
(mean score)		
Vulval irritation	2.11	2.19
Vaginal irritation	2.06	2.13
Discharge	2.25	2.26
Itching	2.40	2.48
Burning	2.21	2.21
Dyspareunia	1.50	1.35

Results

A total of 115 patients were admitted to the study. Data from 14 patients were considered to be invalid and were excluded from the analysis of efficacy. Consequently the outcome of treatment was analyzed in 101 patients: 48 assigned to the single-dose 500 mg clotrimazole group and 53 treated with 200 mg of clotrimazole daily for 3 days. Data from all 115 patients enrolled in the study were included in the analysis of safety.

Baseline data, such as demographic characteristics, pregnancy status, contraceptive use, recent use of other medication, concomitant use of medication other than the study drugs, and disease duration, were comparable in the two treatment groups. The pretreatment severity of the signs and symptoms of vulvovaginal candidiasis did not differ significantly between these two groups (Table I).

Ultimately the number of patients actively enrolled in the study for efficacy were 48 in the 500 mg group and 53 in the 200 mg group. Results of microscopic

Table II. Overall posttreatment response

		5		Clotrii	nazole		·	
		5-10	days			≥27	days	
. ,	500 (n =		$\begin{array}{c} 200 \ mg \\ (n = 5) \end{array}$			$\begin{array}{c} 00 \ mg \\ = 48) \end{array}$	200 (n =	
Response	No.	%	No.	%	No.	%	No.	%
Success . Failure	37 11 .	77 23	47 6	89 11	31 17	65 35	39 14	74 26

examination at the final visit were negative in 39 of 48 patients (81%) treated with a single dose of 500 mg of clotrimazole and in 45 of 53 patients (85%) receiving clotrimazole for 3 days. Cultures at the final visit were negative in 32 of 48 patients (67%) receiving 500 mg of clotrimazole and in 40 of 53 patients (75%) receiving 200 mg of clotrimazole for 3 days. Both microscopic evaluations and cultures were negative in 32 of 48 patients (67%) receiving 500 mg of clotrimazole versus 39 of 53 patients (74%) receiving clotrimazole over 3 days. The results of mycologic tests did not differ significantly between the two groups.

At the first follow-up visit 5 to 10 days after treatment, success was achieved in 37 of 48 patients (77%) treated with a single dose of clotrimazole and in 48 of 53 patients (89%) treated in a 3-day regimen. At the final visit, at least 27 days after treatment, clotrimazole therapy was considered to be successful in 31 of 48 patients (65%) treated with 500 mg and in 39 of 53 patients (74%) treated with 200 mg daily for 3 days. The higher rate of success at the first follow-up visit was attributed to the successful response initially noted in six patients treated with a single dose of clotrimazole, and results in eight patients given the drug for 3 days did not differ significantly at either follow-up evaluation (Table II).

Only three adverse reactions were noted during the study. A vulvar lesion was noted in one patient treated with a single dose of 500 mg of clotrimazole and in one patient treated with 3-day therapy. In both cases, this finding was considered to be remotely related to clotrimazole treatment. A perianal rash possibly related to clotrimazole administration occurred in one patient in the 200 mg treatment group.

Comment

The results of this study, in which administration of a single vaginal tablet containing 500 mg of clotrimazole was shown to be equivalent to treatment with two 100 mg tablets administered over 3 days, support the findings of in vitro investigations with the lactic acid formulation of the drug.

An earlier study compared the vaginal secretion

levels of clotrimazole in women undergoing treatment for vulvovaginal candidiasis after administration of 100 mg for 6 days, 200 mg for 3 days, and 500 mg for 1 day. Secretions were collected at 24, 48, and 72 hours after the last dose of clotrimazole. The vaginal concentration of the drug was determined by measuring the diameter of the zone of inhibition that resulted after introduction of the collected secretions on agar plates inoculated with C. albicans. At all three time periods, vaginal secretions from each treatment group markedly inhibited yeast growth. The zone of inhibition produced by secretions from women treated with the 500 mg dose was consistently greater, however, than that produced by the secretions from women treated with the 100 or 200 mg dose (Fig. 1). These observations demonstrate that levels of clotrimazole achieved after treatment with a single dose of the lactic acid formulation of the drug remain sufficient to inhibit the growth of C. albicans for at least 3 days after administration. Additionally, it appears that the 500 mg, one-dose application can be used alternately in recurrent cases.

With the low water solubility of clotrimazole, tissue penetration of neutral forms of the drug is poor. At a pH of 3.5 to 3.8 in the lactic acid formulation, the release rate of clotrimazole is increased, thereby permitting increased tissue penetration. In addition, animal studies have suggested clotrimazole to be reversibly bound to keratin of the vaginal skin, which may also explain the persistence of high concentrations of the drug several days after administration. Finally, the adhesion of *C. albicans* to the vaginal epithelial cells is markedly stronger and higher at a pH of 6 than at a pH of 3 to 4. All of these features seem to be associated with lactic acid formation and may contribute to the efficacy of 1-day treatment with high-dose clotrimazole.

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Therapeutic results obtained in vaginal mycoses after singledose treatment with 500 mg clotrimazole vaginal tablets

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A multicenter, double-blind study of 103 patients with clinically and mycologically documented vulvovaginal candidiasis compared single-dose treatment with a 500 ng clotrimazole vaginal tablet to 3-day treatment with two 100 mg clotrimazole vaginal tablets administered daily. Patients were examined 5 to 10 days (visit 2) and at least 27 days (visit 3) post treatment. At visit a rrycologic and clinical examinations were negative in 43 of 48 efficacy-evaluable patients receiving cotrimazole, 500 mg (90%), versus 42 of 47 efficacy-evaluable patients receiving clotrimazole, 200 mg (39%). Similarly, at visit 3, 75% of patients receiving clotrimazole, 500 mg, had treatment success versus 72% receiving clotrimazole, 200 mg. There were no significant intergroup treatment differences, indi-ating that single-dose treatment with clotrimazole, 500 mg, is equipotent to the multidose regimen. (AM J CBSTET GYNECOL 1985;152:968.)

Key words: Vulvovaginal candidiasis, clotrimazol-

Patient compliance is an important factor in the successful management of vulvovaginal candidiasis. Few women with this condition delay in consulting a physician for treatment of the clinical signs and sympoms of vaginal mycoses. Once these signs and sympoms subside, however, patients often neglect to continue treatment.

The introduction of the imidazole antifungal agent clotrimazole has had a significant impact on patient compliance. Before the availability of this drug, the required duration of therapy in patients with vulvo-vaginal candidiasis was at least 14 days. Because on the potent antimycotic properties of clotrimazole, this crug was effective when administered once daily for only 6 to 7 days.³⁻⁵

Subsequent investigations showed that the clotrinazole treatment regimen could be further simplified by the use of higher doses of the drug. When a cai y dose of 200 mg was administered, the treatment period could be shortened to only 3 days. 68 The use of this dose level was found to be associated with a higher rare

of patient compliance. Rausch et al. reported a 100% compliance rate in patients treated with clotrimazole for 3 days. In addition, 81% of these patients returned for a follow-up examination. When a 6-day clotrimazole regimen was used, the compliance rate was 94%, and 79% of the patients returned for a follow-up examination. A 12-day clotrimazole regimen was associated with a compliance rate of 83%. Only 66% of patients assigned to this regimen returned for a follow-up examination.

These findings illustrate the relationship between patient compliance and the complexity of the treatment regimen. In an effort to further increase compliance in patients with vulvovaginal candidiasis, a more potent clotrimazole preparation was developed. This formulation contains 500 mg of the drug and is intended for administration only once. A double-blind multicenter trial was conducted to determine whether a single dose of this new preparation was as effective and safe as two 100 mg clotrimazole tablets administered daily for 3 days.

Methods and material

To be eligible for participation in this study, patients were required to be at least 18 years of age and to have clinically diagnosed and mycologically confirmed vul-

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vovaginal candidiasis. No women who had been treated with tetracycline or erythromycin within 7 days of the study or who were undergoing treatment with exogenous estrogens were admitted. The use of contraceptive foams, creams, or jellies was another exclusion factor. The use of oral contraceptive drugs, intrauterine devices, and diaphragms, however, was permitted, provided patients met the other study criteria. Patients suspected of being pregnant and those in the first trimester of pregnancy were not permitted to participate. Also excluded were patients with hypersensitivity to the imidazoles, a history of diabetes mellitus, and concomitant vaginal infections caused by *Trichomonas*, *Gardnerella vaginalis*, or group B *Streptococcus*.

A physical examination was conducted at the initial patient visit, and the degree of vulval irritation, vaginal irritation, discharge, pruritus, burning, and dyspareunia was graded on a scale of zero to three (0 = none, 1 = mild, 2 = moderate; 3 = severe). For the patient to be admitted to the study, a total score for these signs and symptoms of at least three was required. Vaginal specimens for microscopic evaluation (potassium hydroxide) and culture were also collected at this time. The appearance of branching filaments and blastospores on microscopic examination and the growth of Candida species on cultures were considered to provide mycologic evidence of vulvovaginal candidiasis.

After being informed of the nature and purpose of the investigation, study participants were randomly assigned to 1-day treatment with a vaginal tablet containing 500 mg of clotrimazole or to treatment with two 100 mg tablets of clotrimazole daily for 3 days. The medication for the first day of treatment (visit 1) was inserted into the vagina by the investigator. In the single-dose treatment group, one 500 mg clotrimazole vaginal tablet and two placebo tablets identical in appearance to the 100 mg clotrimazole tablets were inserted. These women were then given 100 mg placebo tablets for the remaining 2 days of treatment and were informed how and when to insert them. Similarly, two 100 mg clotrimazole tablets and one placebo tablet identical in appearance to the 500 mg clotrimazole tablet were inserted into the vagina of women assigned to the 3-day regimen during visit 1. These women were then provided with additional 100 mg clotrimazole tablets for the next 2 days.

Follow-up examinations for the signs and symptoms of candidiasis and mycologic assessment were conducted 5 to 10 days after treatment (visit 2) and again at least 27 days post treatment (visit 3). At these visits, patients were also questioned regarding the occurrence of adverse effects.

The overall efficacy of treatment was evaluated by the investigator at visits 2 and 3. Treatment was considered to be successful if the signs and symptoms of

Table I. Demographic and medical characteristics

* .,	Clotre	mazole
	500 mg $(n = 48)$	$200 \text{ mg} \ (n = 47)$
Age (yr)		
Mean	33.0	29.5
Range	19.0-50.0	17.0-51.0
Weight (pounds)		
Mean	140.0	143.7
Range	108.0-214.0	105.0-254.0
Disease duration* (mo)		
0-1	42	44
>1, <6	5	3
Pregnancy status		
Pregnant (>13 wk)	3	1
Nonpregnant	45	46
Method of contracep-		
tion†		
None	19	13
Oral contraceptive	9	20
Intrauterine device	1	1
Other	15	12
Recent use of medication		
Yes	8	10
No	40	37
Concomitant therapy		
Yes	0	1
No	48	46

^{*}Information on disease duration unavailable for one patient in the single-dose group.

vulvovaginal candidiasis had resolved and both microscopic examination and culture of vaginal specimens had negative results. If the clinical signs and symptoms persisted or if either of the mycologic tests were positive, treatment was considered to be a failure.

Baseline demographic and clinical characteristics of patients assigned to the single-day and the 3-day regimens were analyzed with the use of Fisher's exact test for categorical data and Student's *t* test for continuous data. Wilcoxon's rank test was used to determine the pretreatment comparability of signs and symptoms of vulvovaginal candidiasis in the two groups. The response to treatment was compared by means of Fisher's exact test.

Results

This study was conducted at three clinical sites. All investigators followed a common protocol, and the results were pooled for statistical analysis. The three investigators entered a total of 103 patients into the study. Fifty-three of these patients were assigned to the 1-day 500 mg clotrimazole treatment group, and 50 were assigned to the 3-day 200 mg clotrimazole group. All of these patients were included in the analysis of the safety data, but efficacy data from eight patients were consid-

[†]Information on method of contraception unavailable for four patients in the single-dose group and one patient treated with the 3-day regimen.

ered to be invalid. Consequently, the analysis of efficacy was based on results in 95 patients: 48 treated with a single dose of 500 mg of clotrimazole and 47 given two 100 mg clotrimazole vaginal tablets daily for 3 days.

The medical and demographic characteristics of the 95 patients included in the determination of drug efficacy are shown in Table I. Although the group treated with 1-day 500 mg dose of clotrimazole was other (p = 0.05) than the group treated with the 3-day regimen, the other demographic characteristics were similar in the two treatment groups.

Microscopic evaluation was negative at the final patient visit in 40 of the 48 patients (83%) treated for 1 day and 41 of the 47 patients (87%) who received alotrimazole for 3 days. Thirty-nine patients (81%) given 1-day treatment and 39 patients (83%) assigned to reatment with clotrimazole for 3 days had negative cultures. Both mycologic tests—microscopic examinations and cultures—were negative in 38 patients (79%) in the 500 mg group and in 35 patients (74%) treated with 200 mg daily for 3 days. There were no significant mergroup differences in any of the mycologic parameters.

When patients were evaluated 5 to 10 days after reatment, the criteria for a successful response—negative mycologic tests and the absence of clinical sign: and symptoms—were met in 43 patients (90%) treated for 1 day and in 42 patients (89%) treated for 3 days. At visit 3 (27 days or more after treatment), seven of the patients treated with a single dose of clotrimazole and eight treated with the 3-day regimen, in whom reatment was classified as successful at the first follow-up visit, were now categorized as having had treatment failures. Consequently, the success rate at the final sisit was 75% (36 patients) in the 500 mg, single-dose clotrimazole group and 72% (34 patients) in the group treated with two 100 mg tablets daily for 3 days.

Of the 103 patients included in the assessmen of safety, only one, a patient treated with clotrimazoe for 3 days, experienced an adverse reaction. This werran developed moderate edema of the vulva, which was classified as remotely related to the administration of the drug.

Comment

The efficacy and safety of the 500 mg dosage formulation of clotrimazole have also been demonstrated in earlier clinical investigations. In one open-label, multicenter study, the efficacy of a single dose of 500 mg of clotrimazole was evaluated in 178 patients with vaginal mycoses. Candida species were identified at the causative organism in 95% of these patients. A mycologic cure was achieved in 89% of the treated patients, and clinical symptoms resolved in 90%. Mild side effects were reported by seven patients.

Another open-label trial conducted at three gyne-cologic clinics studied the effects of 1-day 500 mg clotrimazole treatment in 90 patients with *Candida* vaginitis. Cultures were negative for *Candida* species in 88% of these patients 1 week after treatment. Results of the microscopic examination of wet films were similar. Improvement in clinical symptoms paralleled the mycologic findings. No clotrimazole-related side effects were reported in this study.

In a double-blind investigation, the effect of a single 500 mg clotrimazole pessary was compared with that of a 200 mg pessary inserted once daily for 3 days in 72 patients with vaginal candidiasis. ¹⁰ One week after the completion of treatment, signs and symptoms of vaginal candidiasis persisted in only one patient in each treatment group. Cultures for *Candida albicans* were negative at this time in 94% of patients treated with the single clotrimazole dose and in 89% of those given the drug for 3 days.

The findings of these earlier trials and those of the study described in this report demonstrate that single-dose therapy with 500 mg of clotrimazole is as effective as a longer regimen using a lower dosage. The simplified 1-day regimen would be expected to improve patient compliance. In fact, because the investigator can insert the tablet during the initial office visit, this new clotrimazole formulation has the potential of completely eliminating the problem of compliance in patients with vulvovaginal candidiasis.

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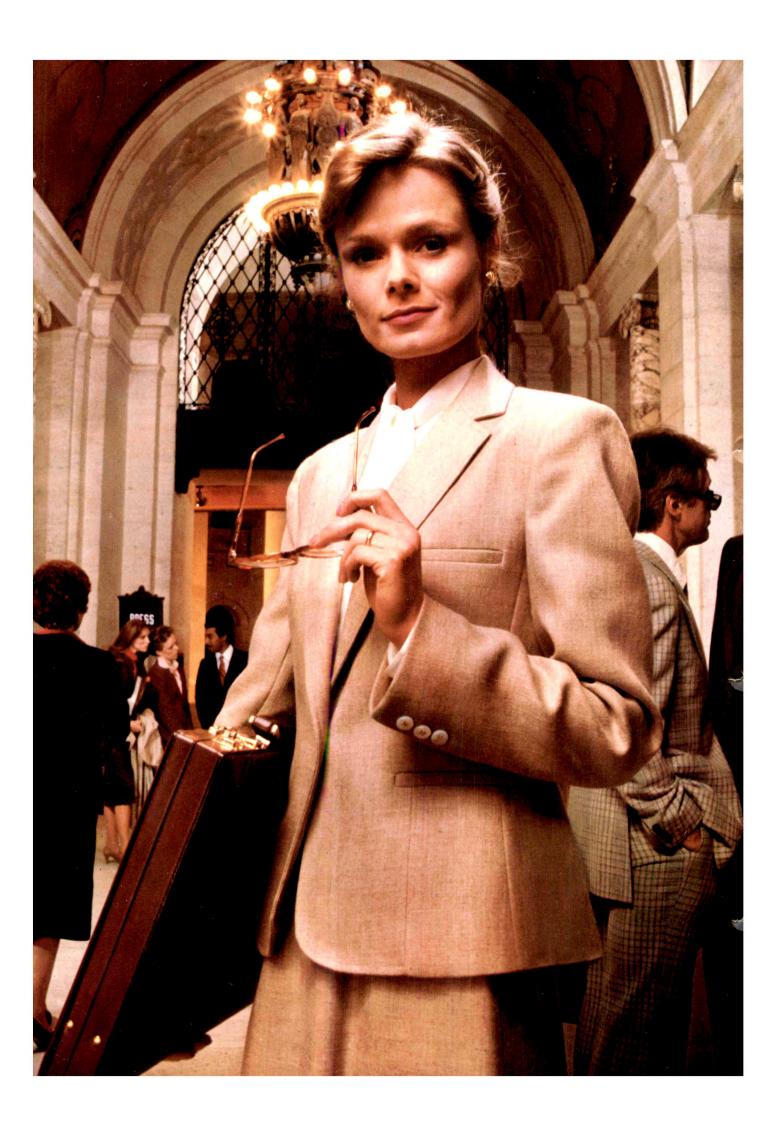
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Indications: Mycelex-G Vaginal Cream is indicated for the local treatment of patients with vulvovaginal candidiasis (moniliasis). As Mycelex-G Vaginal Cream has been shown to be effective only for candidal vulvovaginitis, the diagnosis should be confirmed by KOH smears and/or cultures. Other pathogens commonly associated with vulvovaginitis (Trichomonas and Hemophilus vaginalis) should be ruled out by appropriate laboratory methods.

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Contraindications: Mycelex-G Vaginal Cream is contraindicated in women who have shown hypersensitivity to any of the components of the preparation

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Usage in Pregnancy: While Mycelex-G Vaginal Cream has not been studied in the first trimester of pregnancy, use in the second and third tri.

Cream has not been studied in the first trimester of pregnancy, use in the second and third trimesters has not been associated with ill effects. Application of ¹⁴C-labeled clotrimazole has shown negligible absorption (peak serum level of 0.01 µg/ml 24 hours after insertion of vaginal cream containing 50 mg of active drug) from both normal and inflamed human vaginal mucosa.

Adverse Reactions: Three (0.5%) of the 653 patients treated with Mycelex-G Vaginal Cream reported complaints during therapy that were possibly drug related. Vaginal burning occurred in one patient; erythema, irritation, and burning in another; intercurrent cyclitis was reported in in another; intercurrent cystitis was reported in

the third.

Dosage and Administration: Mycelex-G
Vaginal Cream has been found to be effective
when used from seven to fourteen days; studies
have shown that patients treated for fourteen
days had a significantly higher cure rate. The
recommended dose is one applicatorful a day
for seven to fourteen consecutive days; using the
applicator supplied, insert one applicatorful of
cream (approximately 5 grams) intravaginally
preferably at bedtime. preferably at bedtime

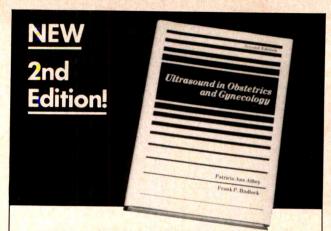
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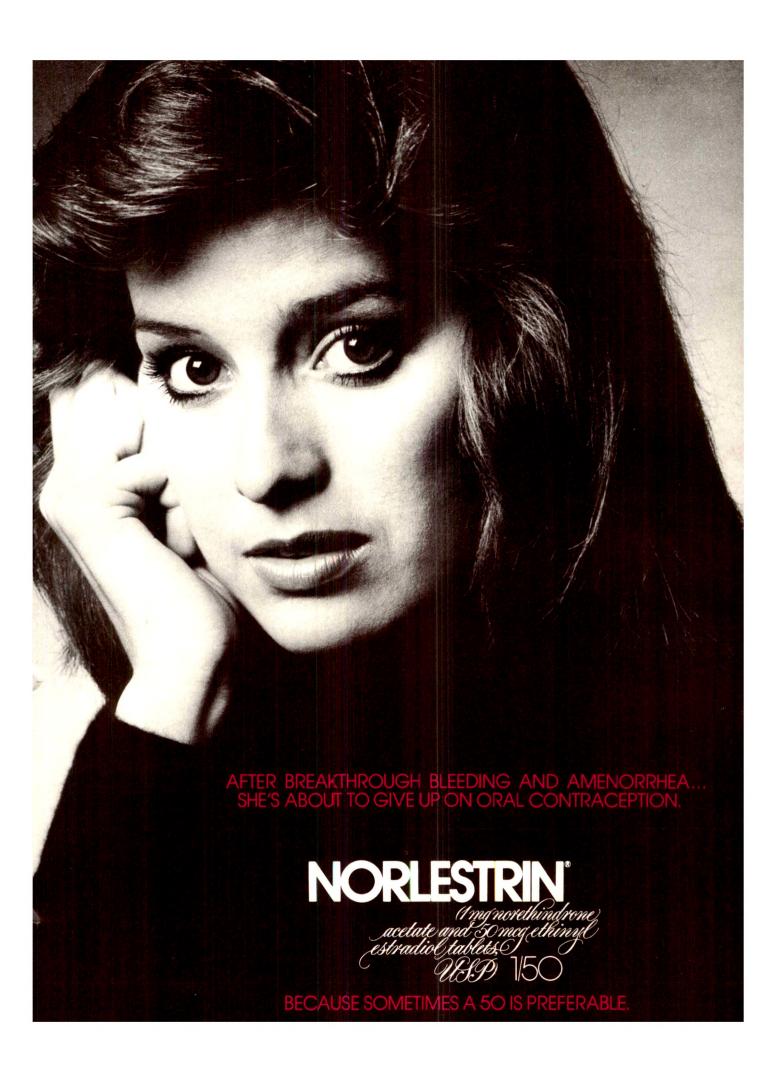
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Brief Summary of Prescribing Information NORLESTRIN® (norethindrone acetate and ethinyl estradiol tablets, USP

See section under **Special Notes on Administration and HOW SUPPLIED.** Before prescribing, please see full prescribing information. A Brief Summary DESCRIPTION

Norlestrin Products are progestogen-estrogen combinations INDICATIONS AND USAGE

INDICATIONS AND USAGE

Nortestrin Products are indicated for the prevention of pregnancy in women who elect to use oral contraceptives as a method of contraception. In clinical trials with Nortestrin 1/50 involving 25,983 therapy cycles, there was a greg nancy rate of 0.05 per 100 woman-years, in clinical trials with Nortestrin 2.5/50 involving, 96,388 cycles, there was a pregnancy rate of 0.22 per 100 woman-years.

Dose-Related Risk of Thromboembolism from Oral Contraceptives: Studies has a Shown a positive association between the dose of extraceptives in the product and the

shown a positive association between the dose of estrogens in oral contraceptives. and the risk of thromboembolism, it is prudent and in keeping with good principles of theragments to minimize exposure to estrogen. The oral contraceptive prescribed for any given paiemt should be that product which contains the least amount of estrogen that is compatised with an accordable preparation to a contains the statement of estrogen that is compatised with an acceptable pregnancy rate and patient acceptance CONTRAINDICATIONS

- 2. Thrombophlebitis or thromboembolic disorders
 2. A past history of deep-vein thromboembolic disorders
 3. Cerebral vascular or coronary artery disease
 4. Known or suspected carcinoma of the breast
 5. Known or suspected estrogen-dependent neoplasia
 6. Undragnosed abnormal genital bleeding
 7. Known or suspected pregnancy (See WARNING No. 5)
 8. Benign or malignant liver tumor which developed during the use of oral contraceptives or other estrogen-containing products.

WARNINGS

Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. The risk increases with age and with heavy smoking of serious cardiovascular side effects from oral contraceptive use. The risk increases with age and with heavy smoking of age women who use oral contraceptives should be strongly advised not to smoke. The use of oral contraceptives is associated with increased risk of several sericular conditions including thromboembolism stroke, invocardial infarction, hepatic active noma, gallbladder disease, and hypertension. Practitioners prescribing oral contacted tives should be familiar with the following information relating to these risks.

**Thrombolish Decorates and Other Remarks Publishers. An increase of tisk of the contact.

nomal galibladder disease and hypertension Practitioners prescribing praticontraceptives should be familiar with the following information relating to these risks.

1. Thromboembolic Disorders and Other Vascular Problems. An increased risk of discrimination of the problems of the problems of the problems of the problems of the problems. An increased risk of distribution of the problems and stroke both hemorrhagic and thrombotic. Cerebrovascular Disorders: In a collaborative study in women with and without pre-hisposing causes, it was estimated that the risk of hemorrhagic stroke was 2.0 times greater in users than nonusers, and the risk of thrombotic stroke was 2.0 times greater in users than nonusers, and the risk of thrombotic stroke was 3.0 to 9.5 times greater. Myocardial Infarction: An increased risk of myocardial infarction associated with challocontraceptives has been reported confirming a previously suspected association. Increased studies found that the greater the number of underlying risk factors (organette smoking) hypertension, hypercholesterolemia, obesity diabetes, history of precediamyte toxical for coronary artery disease, the higher the risk of developing myocardial infarction regardless of whether the patient was an oral contraceptive user or not. Oral contraceptives, heavever, were found to be a clear additional risk factor.

It has been estimated that users who do not smoke (smoking is considered a major predisposing condition to myocardial infarction) are about twice as likely to have a falamity, cardial infarction as nonusers who do not smoke. Oral contraceptive users who are sincipled to be about a fivefold increased risk of tatal infarction compared to users who do not simple but about a tenfold to twelvefold increased risk compared to nonusers who do not simple but about a tenfold to twelvefold increased risk compared to the dose of estrogem is soft oral analysis of data. British investigators concluded that the risk of fire in boembolism including coronary thrombosis is directly relat

Estimate of Excess Mortality from Circulatory Diseases: The risk of diseases of culatory system is cigarette smokers in those with a long duration of use, an £in

cigarette smokers.

A study of available data from a vanety of sources concluded that the mortality assaic afed with all methods of birth control is low and below that associated with chedbirth with the exception of oral contraceptives in women over 40 who smoke. The risk of thromboembolic and hirombotic diseases associated with oral contraemptives increases with age after approximately age 30 and, for myocardial infarction, is further increased by hypertension, hypercholesterolemia, obesity diabetes, or history of pre-eclamptic toxemia, and especially by cigarette smoking. The physician and the patient should be alert to the earnest manifestations of thrombotic disorders. Should any occur or be suspected, the drug should be discontinued immediately.

A fourfold to swidd increased risk of postsurgery thromboembolic complicationsmas been reported in users. If feasible, oral contraceptives should be discontinued at least four weeks before surgery of a type associated with an increased risk of thromboembolic in the prolonged immobilization. prolonged immobilization

Prolonged immobilization

2. Ocular Lesions. Neuro-ocular lesions, such as optic neurilis or retinal thromboiks, have been associated with the use of oral contraceptives. Discontinue the oral contracer, well-there is unexplained sudden or gradual, partial, or complete loss of vision, onser of proptosis or diplopia, papilledema, or retinal vascular lesions.

3. Carcinoma. Long-term continuous administration of estrogen in cenain animal-piece es increases the frequency of carcinoma of the breast cervix, vagina, and liver. In humans, an increased risk of endometrial carcinoma associated with the prolonged use of exogenous estrogen in postmenopausal women has been reported. However, there is no evidence suggesting increased risk of endometrial cancer in users of conventional combination or progestogen-only oral contraceptives. Studies found no evidence of increase in breast cancer in women taking oral compactives, however, an excess risk in users with documented benign breast disease was reported.

tives, however, an excess risk in users with documented benigh breast disease was reported.

There is no confirmed evidence of an increased risk of cancer associated with or incentraceptives. Close clinical surveillance of users is, nevertheless, essential, in cases of and agnostic measures should be taken to rule out malignancy. Women with a strong lamily history of breast-cancer or who have breast nodules, fibrocystic disease, or abnormal maminograms, should be-importanted with particular care.

or who have breast nodules. fbrocystic disease, or abnormal mammograms, should be monitored with particular care.

4. Hepatic Tumors. Being in hepatic adenomas have been found to be associated with oral contraceptives. Because hepatic adenomas may rupture and may cause death through intra-abdominal hemorrhage, they should be considered in women presenting abdominal pain and tenderness, abdominal mass, or shock.

A few cases of hepatocellular carcinoma have been reported in women taking one contraceptives. The relationship of these drugs to this type of malignancy is not known at its stime. 5. Usage in or Immediately Preceding Pregnancy, Brith Defects in Offspring, and Margonacy in Female Offspring. During early pregnancy, female sex hormones may seriously damage the offspring.

An increased insk of congenital anomalies, including heart defects and limb defects has been reported with the use of oral contraceptives in pregnancy.

There is some evidence that triploidy and possible other types of polyploidy are increased among abortuses from women who become pregnant soon after ceasing oral contraceptives.

Pregnancy should be ruled out before continuing an oral contraceptive in any pakentwho as missed two consecutive menstrual periods. If the patient has not adhered to the sched-

ule, the possibility of pregnancy should be considered at the time of the first miss and oral contraceptives should be withheld until pregnancy has been ruled out. If nancy is confirmed, the patient should be apprised of the potential risks to the fett advisability of confinuation of the pregnancy should be discussed.

Women who discontinue oral contraceptives with the intent of becoming pregnancy should be discontinued.

- Women who discontinue oral contraceptives with the intent of becoming pregnuse an alternate form of contraception for a period of time before attempting to condition to a period of time before attempting to condition to the contract of the programment of the p
- Product

 9 Headache: Onset or exacerbation of migraine or development of headache cattern which is recurrent, persistent, or severe, requires discontinuation of oral phraceptives.
- contraceptives

 10. Bleeding Irregulanties. Breakthrough bleeding, spotting, and amenorrhea a quent reasons for patients discontinuing oral contraceptives. In breakthrough ble nonfunctional causes should be borne in mind. In undagnosed abnormal bleedin vagina, adequate diagnostic measures are indicated to rule out pregnancy or ma. Women with a past history of oligomenorrhea or secondary amenorrhea, or you without regular cycles should be advised that they may have a tendency to remail tory or to become amenorrhea after discontinuation of oral contraceptives.

 11. Ectopic Pregnancy Ectopic as well as intrauteme pregnancy may occur in cline failures.

 12. Breast-Feeding. Oral contraceptives may interfere with lactation. Furthermonation of the hormonal agents in oral contraceptives has been identified in the mo-

fraction of the hormonal agents in oral contraceptives has been identified in the m ese druas

PRECAUTIONS

- PRECAUTIONS

 1. A complete medical and 'amily history should be taken prior to the initiation of raceptives. The pretreatment and periodic physical examinations should include reference to blood pressure, breasts, abdomen, and pelvic organs, including Papsmear and refevant laboratory tests. As a general rule, oral contraceptives should prescribed for longer than one year without another examination.

 2. Preexisting uterine leiomyomata may increase in size.

 3. Patients with a history of psychic depression should be carefully observed and discontinued if depression recurs to a serious degree.

 4. Oral contraceptives may cause fluid retention and should be prescribed with and only with carefull monitoring, in patients with conditions which might be aggrated to the prescribed with a past history of jaundice during pregnancy have an increased recurrence of laundice. If jaundice develops, the medication should be discontinued. Siteroid hormones may be poorly metabolized and should be administered with patients with impaired liver function.

 7. Users may have disturbances in normal tryptophan metabolism, which may rerelative pyridoxine deficiency.

- relative pyridoxine deficiency.
 8 Serum folate levels may be depressed.
 9 The pathologist should be advised of oral contraceptive therapy when relevant mens are submitted.
- mens are submitted 10. Certain endocrine and liver function tests and blood components may be affi-(a) Increased sulfobromophihalein retention (b) Increased prothrombin and fac VIII. (X. and X. decreased antifhrombin 3, increased oncep nephrine-induced plate gability (c) Increased thytoid-binding globulin (TBG) leading to increased circulat thytoid bormone. (d) Decreased pregnanediol excretion. (e) Reduced response to

pone lest **Drug Interactions:** Reduced efficacy and increased incidence of breakthrough bhave been associated with concominant use of ritampin. A similar association has tigested with barbiturates, phenylbutazone, phenyloin sodium tetracycline, and am **ADVERSE REACTIONS**. An increased risk of the following serious adverse reactions has been associated a contraceptives: thrombophiebitis: pulmonary embolism, coronary thrombosis, cerifitrombosis, cerebral hemorrhage, hypertension, galibladder disease, benign hep condental anomalies.

contraceptives fromtophieptiss, pulmonary embolism, coronary thrombosis, centrombosis, cerebral hemorrhage, hypertension, gailibladder disease, benigh hep congenital anomalies.

There is evidence of an association between the following conditions and the us contraceptives, although additional confirmatory studies are needed, mesentenor as, heuro-ocular lessons, egi etinat, hrombosis and optic neuritis.

The following adverse reactions have been reported in patients receiving oral collives and are believed to be drug related, nausea and/or vomiting, usually the mostomen adverse reactions, occur in approximately 10% or less of patients during the figure reactions as a general rule, are seen much less frequently or only occasioning astromestimal symptoms, break hrough bleeding, spotting, change in mensitual dysmenormea, amenormea during and after freament, temporary infertility after of timulance of freatment, edema, chloasma or metasma, breast changes, change in whange in corvical erorison and cervical secretion possible diminition in lactation is given immediately postpartium, cholestatic jaundice, migrame, increase in size of upomyomata, rash (altergic) mental depression, reduced folerance to carbohydra vagnal candidiasis, change in conneal curvature, intolerance to contact lenses. The following adverse reactions have been reported and the association has been confirmed nor refuted premenstrual-like syndrome, calariacts, changes in libido, changes in appetite, cystifis-like syndrome, headache, nervousness, dizzness, hir loss of scalp hair erythema multiformo, erythema nodosum, hemorrhagic eruption, porphyria.

Special Notes on Administration

day after discontinuing medication.

After several months on freatment, bleeding may be gin as late as the fourth of the day after discontinuing medication.

After several months on freatment, bleeding may be reduced to a point of writing. After several months on treatment bleeding may be reduced to a point of virtual reduced flow may be a result of medication and not indicative of pregnancy.

HOW SUPPLIED

reduced flow may be a result of medication and not indicative of pregnancy. **HOW SUPPLIED**Norlestrin [21] 1/50 is available in compacts each containing 21 tablets. Each table 1 mg of norethindrone acetate and 50 mcg of ethinyl estratiol. Available in packagr compacts and packages of five refills.

Norlestrin [21] 2 5/50 is available in compacts each containing 21 tablets. Each it contains 2.5 mg of morethindrone acetate and 50 mcg of ethinyl estradiol. Available ages of five compacts and packages of five refills.

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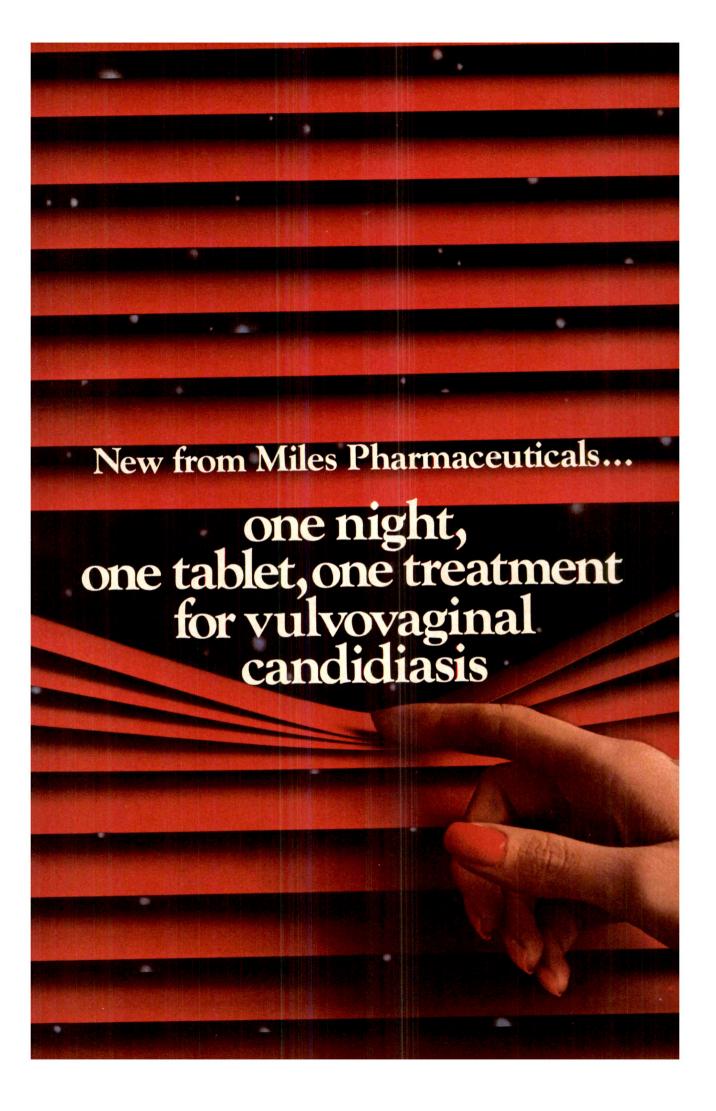
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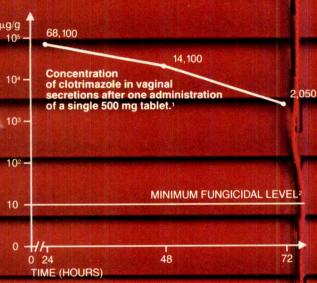
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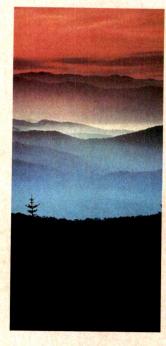
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CLINICAL SECTION

Clinical Opinion

Incisions of the pregnant uterus and delivery of low-birth weight infants

Radoslav Jovanovic, M.D.

New York, New York

This paper calls attention to rapidly increased use of vertical uterine incision and future consequences: impaired uterus, uterine rupture, and iatrogenic premature delivery. In addition, by making a vertical uterine incision we are not gaining much in the size of uterine entrance since the size of the vertical uterine incision is determined by uterine tonus. Most often the uterus is opened by tearing the lower uterine segment transversely with the fingers. Unfortunately this creates the smallest entrance to the uterus because of the uterine structure, shape, and elasticity and could lead to parametrial vein and uterine vessel injury. An alternative to this type of incision is the durable upward curved transverse incision over the supportive lower uterine segment. This technique gives us the biggest entrance to the uterus, without serious side effects, and ensures safe future pregnancies. (AM J OBSTET GYNECOL 1985;152:971-4.)

Key words: Cesarean section, uterine incisions

The continued and rapidly increasing use of vertical uterine incision for cesarean delivery over the last several years is of concern to some of us as clinicians. It appears that obstetricians today more quickly decide to perform vertical uterine incisions and more easily find justification to do so.

The vertical incision can be replaced by an upward concave transverse incision over the low uterine segment without sacrificing the size of the incision.

Vertical uterine incision

The use of a vertical uterine incision for cesarean delivery, once condemned as a weak incision, has rapidly increased over the last several years, causing a concomitant increase in impairment of the uterus which is of considerable concern. It is well known that vertical uterine incision is related to significant maternal and perinatal morbidity and mortality. More importantly, the potentially serious complications associated with vertical incision are difficult to prevent. In addition, all

livered prematurely.

There are, however, occasions when a vertical uterine incision is preferred over a transverse incision. The most common occurrence, and perhaps the only circumstance in which some of us have used a vertical incision, is to deliver an infant from a patient scheduled to have a cesarean hysterectomy. A vertical incision is also preferable for patients in active labor with trans-

patients having a vertical uterine scar must be delivered

by cesarean section before labor, that is, before the

completed term of pregnancy. As a consequence, even

when everything is going well, some infants will be de-

verse lie. There are probably additional rare occasions in obstetrics for which a vertical incision may be the best choice, such as anterior placenta previa and myoma of the low uterine segment.

of the low uterine segment.

Unfortunately, the majority of vertical uterine incisions today are performed for the delivery of a small infant. It was reported in this JOURNAL that cesarean section is the preferred method of delivery for all fetuses weighing under 1000 gm,¹ and suddenly all obstetricians began to follow this dictum without asking if this was really best for the particular infant in the particular circumstance. Obstetricians, overwhelmed

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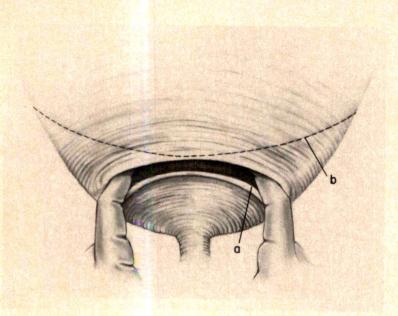


Fig. 1. Comparison of lengths of incisions by two techniques. The length of a transverse incision forcefully extended by blunt tear by fingers is indicated by a. This technique caused a concave downward tear secondary to (1) the circular muscles in the lower uterine segment which directed the tear downward and (2) the elastic quality of the uterine wall which impeded the horizontal extension. The larger concave upward incision created by the Bendage scissors is indicated by b.

by an immediate problem, may disregard the patient's future by making a vertical uterine incision and thus severely limit the number of subsequent pregnancies she can have. In short, it appears that too many vertical uterine incisions are being performed today. By the time this is realized, there will be many women who not only have an impaired uterus but also face the potential disaster that can result from a ruptured scar before delivery.

In the past, obstetricians have learned again and again that a general rule does not always serve as the best guide for an individual patient's obstetric care. As Rosen and Chik2 so aptly stated at the conclusion of a recently published study, "categorical statements such as ones regarding cesarean births for the delivery of the very low-birth weight fetus cannot be justified." By the same token, to completely erase the rule that all small infants should be delivered by cesarean section is simply illogical and irrational. Some small infants in vertex or breech presentation will do better by vaginal delivery and others by cesarean section. The approach for each patient should be individualized, with the obstetricians's clinical judgment being the most important factor in deciding the optimal method of delivery of a small infant. It is now reported that small infants would suffer no greater trauma by vaginal delivery than by cesarean section and would, in fact, do better if delivered vaginally. Several studies support this position.3 Olshan et al.4 have shown that, regardless of presentation, cesarean delivery produced no changes in neonatal mortality for 345 babies weighing 700 to 1500 gm, while studies by Tejani et al.5 and Rayburn et al.6

revealed that abdominal delivery of small infants in breech or vertex presentation did not prevent periventricular and intraventricular hemorrhage.

Labor may actually be beneficial to small infants, since some factor present in labor stimulates the release of lung surfactant from alveolar cells into the alveoli. Vaginal delivery gives small infants sufficient time to mold, and a slow delivery with a big episiotomy is less traumatic than suddenly pushing an infant through a narrow opening for cesarean delivery. In addition, premature infants delivered vaginally are not exposed to the respiratory distress syndrome related to cesarean delivery or to the negative effect from general anesthesia or epidural anesthesia. 10

However, if individualized clinical assessment indicates that a small infant should have an abdominal delivery, then this infant should be delivered through a semilunar upward concave incision over the low uterine segments.

Transverse uterine incision

Usually an obstetrician makes a transverse uterine incision over the low uterine segment by entering the uterus with a knife and then extending the incision laterally with the fingers. By doing so, one is really blindly tearing the low uterine segment, a tear over which one has no control at all. This tear is most probably determined by sphincterlike muscular fibers over the low uterine segment above the cervix and by the elasticity of the uterine wall. This elasticity prevents tearing to the side of the uterus. So created, the finger circular tear is a semilunar downward concave incision

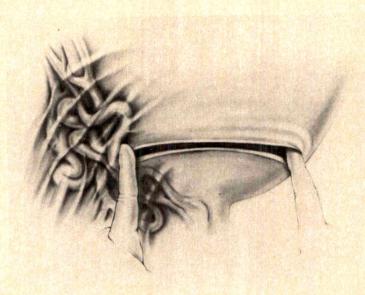


Fig. 2. An attempt to extend a small incision laterally with force by fingers. This figure illustrates the likelihood that the veins could be torn.

over the low narrow pole of the elliptic body presented by the pregnant uterus. More importantly, this finger tear creates the smallest possible transverse incision over the low uterine segment. Fig. 1 illustrates this clearly.

Often during an operation an obstetrician realizes that the transverse incision created by the fingers is too small and attempts to extend the incision laterally with the fingers. In doing this, the fingers can easily slip at the ends of the incision to the parametria, tearing large veins there and possibly leading to serious consequences. Fig. 2 illustrates this problem.

However, the most frequent complication of tearing the uterus transversely with the fingers is not tearing the parametria but involuntarily extending the tear down the lateral side of the uterus during delivery. We have no control over these lateral tears where the largest and most distended uterine vessels are and most probably are predisposed by downward concave finger tears and the structure of the uterus (see Fig. 3).

As a result of this uncontrolled, blind tearing of the low uterine segment, serious complications can occur. One such example was a case in which the ureter was tied in an attempt to control bleeding in the parametria. Another patient received 36 blood transfusions and survived only by luck. A hysterectomy had to be performed to save the life of a third patient. These are only a few of the more drastic problems that have been observed, with a prevalence as high as 1 in 500. There are many more, all caused by use of the fingers to tear the low uterine wall.

It is a simple physical law that the length of an incision is determined by the shape of a cylindrical body and the direction of the incision or tear. Thus a bigger incision can be created transversely if, after the uterus is entered with a knife, we extend the incision as shown in Fig. 1. This incision, an upward concave incision, is not only the biggest transverse incision possible over the low segment, which is not shortened by uterine spasm during surgery, but it is also easy to make and under our control. After entrance into the uterus with a knife, two fingers are placed in the uterus to protect the infant, and the low uterine segment is cut by Bendage scissors transversely and upwardly, close to the sides of the uterus, as illustrated in Fig. 1. This upward concave incision gives us enough room for the delivery of either a small or a large infant from a nonlabored uterus throughout the entire course of pregnancy. After the placenta is delivered and the uterus begins to contract this incision becomes straight transverse. The quality of this incision is good, since it is not over the most active part of the uterus but rather over the supporting part of the low uterine segment, which widens as uterine size and sensitivity increase during pregnancy.

From a practical point of view the size of the low uterine segment can be appreciated in the third trimester. Some patients have a wider low uterine segment, related to the position of the presenting part and to uterine sensitivity, when not in labor than others do in labor. Also a patient may have a wider low uterine segment early in the third trimester than another patient at term. Unlike the size of a vertical incision made over the most active part of the uterus, the size of a transverse incision over the supporting or less active part of the uterus is not affected during surgery. When delivering an infant through a vertical incision, the uterine spasm must be overcome, which sometimes requires considerable force.

The proportion of smooth muscle in the myome-



Fig. 3. A schematic representation of the lateral extension of the concave downward finger tear during delivery. The downward pressure of the presenting part extends this tear in the direction of least resistance, which is the lateral uterine wall.

trium decreases from the fundus to the cervix. About 65% to 70% of the myometrium is smooth muscle in the fundus, and only 25% is in the upper cervical segment. In contrast, connective tissue of the uterus increases proportionately toward the cervix, which is made up of collagenous tissue by more than 80%.

It appears that the uterine structure is such that weaker points are in a longitudinal direction and that is why the most common type of tear in the unscarred uterus is a longitudinal one. Moreover, the strong structure is in the transverse direction of the lower part of the uterus so that even a dehiscent transverse scar sustains labor, and sometimes delivery, well. Certainly these uterine qualities also suggest that a transverse incision would be stronger.

The upward concave incision over the low uterine segment has been tested on many patients in the past, but it has never been reported to be less durable than a transverse incision created by the fingers. Not only is this upward concave incision adequate for delivering an infant of any size, but it is also safe. Parametrial vein injury is virtually nonexistent, and the rare extensions that do occur are most often over the middle part of the low uterine segment, an area that is safe to repair because it contains neither the ureter nor large blood vessels. When the low transverse incision is compared with the low vertical incision, the former is preferable, according to a study by Schutterman and Grimes15 which demonstrates that "low transverse incision for cesarean delivery of breech infants has a short-term safety similar to low vertical incision as well as an important long-term benefit . . . the option of subsequent vaginal delivery."

Comment

Clinicians will provide better obstetric care by relying more on their clinical judgment and less on rules made up by "overambitious and prestigious" physicians.

This review supports the opinion that weak and spastic vertical incisions can be replaced by durable upwardcurved transverse incisions without compromising the size of the uterine entrance, without serious side effects, and with safer future pregnancies.

Thus the fingers should never be used to tear the pregnant uterus transversely, since this creates the smallest possible transverse incision and can lead to serious maternal injury.

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Clinical Articles

Weight-specific stillbirths and associated causes of death: An analysis of 765 stillbirths

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An analysis of 765 consecutive stillbirths associated with 98,927 pregnancies during a 6-year interval showed significant differences for cause of death at specific weight categories. In addition, 57% of stillbirths occurred at infant weights of ≥1500 gm. Hypoxia accounted for 43% of all stillbirths. The implications with respect to preventability and for changes in routine prenatal care through the incorporation of the several methods of fetal assessment currently available are discussed. (AM J OBSTET GYNECOL 1985;152:975-80.)

Key words: Causes of hypoxic stillbirths, prevention, third-trimester prenatal strategies, ultrasound

Reviews of perinatal mortality and their statistical analysis are accepted methods of measuring fetal outcome. Identification of specific causes of death may allow preventative or corrective action that could lead to a reduction in mortality. A useful review of perinatal mortality, particularly if specific subgroups are subject to analysis, requires a large series with some assurance that these deaths are derived from a representative sampling of the general obstetric population. These conditions are most likely to be met by an examination of regional perinatal deaths, such as the United States Collaborative Perinatal Project' and the British Perinatal Mortality Survey.2 However, both these studies were carried out approximately two decades ago. Since that time there has been a greater reduction in neonatal deaths than in stillbirths. The latter still outnumber neonatal deaths.3 Because more recent studies have reported a limited number of deaths, usually from an institution, and have not always used a clinically useful classification, it is still difficult to identify specific strategies that might reduce stillbirths.

The major objective of this study was to review causes of stillbirth assigned to specific weight categories to de-

termine what differences might be present and from this analysis to determine whether the potential existed for the reduction of stillbirths. Secondary objectives were to determine intraregional disparities, if any, and to assess the consistency of the classification used in this system.

Methods and material

The total number of confinements in our province from 20 weeks' gestation during a 6-year period from 1977 to 1982 was 98,927. The resulting 765 stillbirths are the subject of this analysis. During this time there were no significant changes in the distribution of obstetric care. The Perinatal and Maternal Welfare Committee of the College of Physicians and Surgeons of Manitoba receives notification of stillbirths for the ent re region from three independent sources and obtains information for analysis from the stillbirth certificate, the medical chart, correspondence with the attending physician, and the autopsy report and through the perinatal committees of the six larger hospitals. The circumstances surrounding each stillbirth are examined in detail by two individuals in an attempt to identify factors that may have contributed to the death. The coding and classification of all deaths were performed by the same two individuals throughout the review period. While this process is obviously retrospective for each individual death, the collective coding and classification were prospective since each single review was carried out without knowledge of the final annual total of stillbirths or the causes of death.

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Table I. Classification of cause of stillbirth

Hypoxia Subcategory	Criteria
Intrauterine growth retardation	Birth weight <10th pecentile for gestation and gesider with no congenital assomalies. With meconium present in vertex presensition and/or autopsy evidence of hypoxia, or poor mastrnal weight gain <10 pounds associated with symphsis fundal height measu ements
Cord accidents/ compression	Nuchal cord ≥2, or tru± knot, or prolapse, or perfcation at amniocentesis
Maternal hypertension	Pregnancy-induced hypertension—essential, preedampsia. Prenatal recordings of blood pressure ≥ €0/90 mm Hg on at least coccasions and/or intr. partum hypertension ≥ 1±0/100 mm Hg
Placental insufficiency	Autopsy evidence of hymoxia with appropriate weight for gestation. With meconium or meconium-sained membranes in vertex presentation; or birth weight/placental weight ratio >7:1 or placent infarcts >25%
Postmaturity	≥42 wk, with some confirmation of gestation where possible (ultrasound, pregnancy test). Meconium or autopsy evidence o hypoxia or meconium astiration
Other	Low birth weight for gesætion, unspecified, with the presence or absence ·? anomalies not known. Eirth weight <1000 gm with intrapartum death. Altormal cord insertion, vasa previa, or prolonged labor

The system of classification for cause of death _sed in this review was based on the clinical-pathologicxlassification of the British Perinatal Mortality Survey and was chosen prospectively 7 years ago prior to the collection of this material. In classifying deaths only the major contributing cause of death was selected although in many instances there were multiple facors. Unless the case is particularly clear-cut there is all ays an element of judgment necessary in choosing one single factor as the major cause of death. For exampe, a stillbirth that occurred in a patient with gestational diabetes as a result of a major degree of abruptio placentae at 34 weeks' gestation would be classified under the category of antepartum hemorrhage, abruptio placentae. The general precept that was followed in the

Table II. Distribution of stillbirths by weight-specific subgroups

Weight category (gm)	n	% of total
<1500	330	43.1
1500-2499 ⁻	172	22.5
≥2500	263	34.4
Total	765	100

classification process is best illustrated with a quotation from Baird and Thompson⁴ in the British Perinatal Survey, "... it is better, for the purposes of analysis, to demand reasonable probability than to insist on complete certainty."

The following major groups were used for classification of stillbirth: hypoxia, antepartum hemorrhage, congenital anomalies incompatible with life, diabetes, trauma, miscellaneous conditions, and unclassified cases. Under the major category of hypoxia, there were six subcategories: intrauterine growth retardation, cord accidents/compression, maternal hypertension, placenta insufficiency, postmaturity and "other." These are defined in Table I. Wherever possible, deaths were assigned to each of these subcategories of hypoxia according to several criteria in an attempt to increase the accuracy of case assignment. For example, it was felt that the diagnosis of true intrauterine growth retardation was strengthened if associated with some clinical or pathologic evidence of hypoxia or impaired clinical growth. Similarly, the recording of two episodes of increased blood pressure often separated by an interval of several weeks during an otherwise apparently normal prenatal course was felt inadequate as a definition of significant maternal hypertension. Again, it is often difficult to distinguish the difference between an unclassified stillbirth and placental insufficiency leading to hypoxia without criteria that might demonstrate an abnormally small placenta in relation to birth weight or disruption of a significant area of the placental surface, arbitrarily chosen as 25%.

Antepartum hemorrhage consisted of three subcategories: major abruptio placentae, placenta previa, and significant unexplained antepartum hemorrhage. The majority of stillbirths in this category occurred in association with major abruptio placentae and classification was generally not difficult since there was usually sufficient clinical evidence, including the examination of the placenta, to assign the case to this category.

Congenital anomalies incompatible with life were usually not a problem in assignment of cause of death. These cases were always assigned to this category regardless of any other associated conditions.

The diagnosis of diabetes was, for the most part,

Table III. Major categories of causes of stillbirths for weight-specific subgroups

•		Weight	•		
Category	<1500 gm (n)	≥1500-2499 gm (n)	≥2500 gm (n)	n	%*
Hypoxia	126	84	121	331	43
Antepartum hemorrhage	70†	. 22	26	118	16
Congenital anomalies	39‡	22	17	78	10
Diabetes	1	5	31§	37	5
Miscellaneous	26†	6	8	40	5
Trauma	4	l	8	13	2
Unclassified	64	32	52	148	19
Total	330	172	263	765	

^{*}Percentages rounded out.

based on serum glucose values and included insulindependent and gestational diabetes. Because of a lack of prenatal blood glucose values in some instances, stillbirths were assigned to this category because of the patient's family and past obstetric history associated with a stillborn infant exceeding 4000 gm in weight, since these factors are considered to increase the possibility of gestational diabetes, or because of the findings at autopsy with respect to the fetal pancreas.

The miscellaneous category included stillbirths arising from conditions such as cholestatic jaundice or maternal anticoagulation; stillbirths secondary to hemolytic disease, particularly Rh isoimmunization; bacterial causes associated with prolonged rupture of the membranes or viral infections such as cytomegalovirus; and other causes such as intra-abdominal pregnancies or automobile accidents. Cases were considered as unclassifiable when it was not possible to explain the stillbirth on the basis of any of the above conditions or when the evidence seemed insufficient to explain a major occurrence of this nature.

Once coding was completed the information was entered and stored on a Phillips Model M2001 word processor. The χ^2 test with the Yates correction was used for statistical analysis.

Results

There were 765 stillbirths associated with 98,927 pregnancies from 1977 to 1982 for a stillbirth rate of 7.7/1000. The corrected stillbirth rate was 6.0/1000. The overall autopsy rate was 80%, varying between 74% and 90% on an annual basis. Table II shows the distribution of these stillbirths by weight-specific subgroups. At least one third of the total number of stillbirths occurred at infant weights ≥2500 gm.

Table III shows the causes of death for stillbirths in these three weight-specific groups. The largest single major category associated with stillbirth was hypoxia (43%). Next was the group coded as unclassified, for which a cause of death could not be determined with a reasonable degree of probability (19%). It can be seen from Table III that the distributions for some of the causes of death varied significantly with the weight group. For example, antepartum hemorrhage, which in the majority of instances consists of abruptio placentae, accounted for a significantly greater number of stillbirths at infant weights of <1500 gm than for stillbirths at infant weights of >2500 gm (p < 0.001). Similarly significant differences were shown for diabetes and lethal congenital anomalies. Seventy-eight percent of stillbirths with lethal anomalies occurred at infant weights of <2500 gm (61 of 78). The proportion of unclassified cases for each weight subgroup was very

Since hypoxia was a cause of death for 43% of all stillbirths the subgroups within the category of hypoxia were examined in more detail (Table IV). The weight distribution of intrauterine growth retardation and postmaturity was affected by the definitions used for these subgroups and statistical significance was not relevant. The greater number of stillbirths <1500 gm shown in the "other" category again probably reflects the method of classifying these conditions collectively since many of the causes occurred infrequently and sporadically as separate events (Table I). The single largest component of hypoxic stillbirths was intrauterine growth retardation (26%). During a 6-year period only nine stillbirths of infants that weighed ≥2500 gm were less than the tenth percentile. Of cord accidents, 47% (29 of 61) occurred in the mature weight group ($\geq 2500 \text{ gm}$).

Table V shows selected causes of death expressed as a percentage of the total number of stillbirths compared to the percentages when the denominator was changed

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tp < 0.001 (<1500 versus ≥ 2500 gm).

p < 0.05 (<1500 versus ≥ 2500 gm).

p < 0.01 (<2500 versus ≥ 2500 gm).

Table IV. Categories of hypoxia for weight-specific subgroups of stillbirths

-	·	Weight	•		
Category	<1500 gm (n)	1500-2499 gm (n)	≥2500 gm . (n)	n	.%
Intrauterine growth retardation	37	40	9	86	26
Cord accidents/compression	21	11	29	61	18
Maternal hypertension	20	16	21	57	17
Placental insufficiency	13	15	-29	57	17
Postmature (≥42 wk)	0	0 .	28	28	9
Other	35	2	5	42	13
Total	126	84	121	331	

Table V. Stillbirths for selected categories expressed as a percentage of total stillbirths and compared with weight-specific and cause-specific subgroups

	% Total	V 1	cific subgroups ıly	% Weight-spec specific subgro	ific and cause- ups—Hypoxia
Category	stillbirths	<2500 gm	≥2500 gm	<2500 gm	≥2500 gni
Postmature	. 3.6		10.6	_	23
Intrauterine growth retardation	11.3	15.3	_	36	_
Diabetes	4.8		11.8	_	
Antepartum hemorrhage	15.4	18.3			_

Numbers and totals shown in Tables III and IV.

by considering first a specific weight category and then, in addition, a specific cause of death such as hypoxia. It can be observed that while postmaturity accounted for only 3.6% of the total number of stillbirths the middle column in Table V shows that this increased to 10.6% of the deaths in the weight group >2500 gm (28 of 263). The third column in Table V shows that postmature stillbirths accounted for 23% of the total ir. the weight-specific group >2500 gm and the cause-specific group of hypoxia (28 of 121, Table IV). In a similar manner Table V illustrates the changing proportions for intrauterine growth retardation, diabetes, and antepartum hemorrhage when the analysis takes into account weight-specific and cause-specific subgroups when appropriate. These are examples that represent a more appropriate method of analysis for determining the relative importance of certain causes of stillbirth, particularly in defining areas of preventability.

No significant differences emerged on analysis of the results between the metropolitan area and the rural. With the distribution of stillbirths by weight-specific categories, as shown in Table II, 62% of rural stillborn infants were ≥1500 gm compared to 57% of metropolitan stillborn infants. The percentages of stillbirths secondary to hypoxia, congenital anomalies, diabetes, and the unclassified cases were similar for both urban and rural areas. Traumatic stillbirths showed a difference, particularly in infant weights <2500 gm, with a higher percentage attributed to the rural area, but the

numbers are too small, with a total of 13 cases for 6 years, to justify comparison (Table III).

Unclassified cases accounted for 20% of stillbirths for 4 consecutive years with 17% and 19% for the first and last years respectively.

Comment

The object of this analysis was to review the causes of stillbirth and their weight categories in an attempt to define areas of preventability. It is generally accepted that neonatal deaths in infants ≥1500 gm are preventable.5 In contrast, in this series, 57% of stillborn infants weighed >1500 gm and 34% weighed >2500 gm. A similar distribution has been reported for fetal deaths before labor with 46% of deaths at infant weights of ≥2500 gm and 78% at infant weights of ≥1500 gm.6 This suggests that there is a significant potential for the reduction of stillbirths. Both the cause of death and the weight category of stillbirths must be available for analysis if these areas of preventability are to be defined more precisely. The Collaborative Project recorded the events surrounding the confinement of 53,518 pregnancies and their outcomes, with an autopsy rate of 72%. This review examines the 765 consecutive stillbirths associated with 98,927 confinements from one region, with an autopsy rate of 80%.

Of the selected categories in this classification hypoxia was designated the major cause of stillbirth for 43% of the cases (Table III). Intrauterine growth re-

tardation accounted for 26% of hypoxia-related stillbirths (Table IV). This condition accounted for an even greater proportion of stillbirths (36%) if the weightspecific category was also considered in relation to hypoxia as the cause of death (Table V). While the diagnosis of intrauterine growth retardation should be made clinically, this condition is not always easy to detect and perhaps the functional assessment of fetal wellbeing and the estimation of amniotic fluid volume by ultrasound should be done routinely in the early third trimester to detect unsuspected cases of intrauterine growth retardation.7.8

Placental insufficiency in the absence of overt maternal disease or intrauterine growth retardation accounts for 17% of hypoxia-related stillbirths. Fifty percent of these deaths (29 of 57) occur at infant weights ≥2500 mg (Table IV). Clinically this has always been an unsatisfactory diagnosis and almost invariably retrospective. However, the routine use of fetal movement counts in the third trimester as a standard prenatal strategy is worth consideration. Several authors 9, 10 have advocated the use of fetal movement counts; despite this, it remains an optional feature of prenatal care. Used in conjunction with ultrasound fetal assessment when indicated, it has the potential to reduce the incidence of stillbirths.

The management of postmaturity remains controversial with the major issue being the problem of accurate dating of the pregnancy. One solution to this problem requires the early and accurate dating of each pregnancy, which implies routine ultrasound scanning for dating prior to 20 weeks.

Table IV, which lists the various subcategories for hypoxia, shows that 29 of 61 stillbirths due to cord accidents/compression occurred at birth weights ≥2500 gm (47%). While cord accidents have generally been considered preventable it might be worth reconsidering this nihilistic attitude. The diagnosis by ultrasound of persistent occult cord presentation has been recently described." The question arises whether increased efforts to detect occult cord presentations with ultrasound, particularly in breech presentations or unstable lies, might alter the management and prevent some stillbirths from this cause.

These and other strategies, many of which have been reported in the past, if implemented as an accepted and routine component of prenatal care in the third trimester, have the potential to reduce hypoxic stillbirths, which for the most part are the most amenable to prevention. It should be emphasized that all stillbirths in this category occurred in normal babies without major congenital anomalies incompatible with life. In contrast, when the other causes of weight-specific stillbirths are reviewed (Table III), the prospects for improving the outcome are poor for categories such as antepartum hemorrhage, congenital anomalies, and miscellaneous conditions. Not only are these conditions nct currently amenable to correction or prevention but they occur at low birth weights, which also influences the prospects for survival. With our current knowledge we would not foresee a major reduction in stillbirths at these weights arising from these conditions. It should, however, be possible to improve the results in diabetic and hypertensive pregnancies with better or improved prenatal care within the current management protocols that are generally accepted for these conditions. It is inceresting to note that for the diabetic patient the potential exists even in the third trimester for improved results since most of these stillbirths occurred at birth weights of ≥2500 gm.

Although any clinical system of classification for perinatal deaths can be criticized because of its arbitrary nature or the element of judgment involved in deciding the major cause of death, it is possible to maintain reascnable consistency. Our figure of 19% for unclassified cases is in agreement with the findings of 20% for the United States Collaborative Project.12 Various other methods of classification have been used in similar studies.13,14 For the clinician the most useful form of classification for analysis is one that in some way uses functional clinical entities that are recognizable in the practize of obstetrics and can therefore be identified prospectively before the occurrence of a stillbirth.

In conclusion it is suggested that the components of routine prenatal care should be more clearly defined to incorporate the several methods of fetal assessment that have been developed in the last decade. Hypoxia as a cause of stillbirth is a category that has the greatest potential for fetal salvage. While not all of these deaths will prove preventable, the third trimester should be a period of intensive surveillance to a greater extent than carrently exists and these efforts should prove more rewarding in reducing stillbirths. Whether this increased effort and expense to achieve a reduction of stillbirths would be as acceptable to the consumer or the fiscal agency responsible for costs is a matter for onjecture. Nevertheless, by defining what our efforts are attempting to achieve at each trimester of pregrancy, we could focus the appropriate medical and f-scal resources where they would have the most €ffect.

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Low birth weight, intrauterine growth retardation, and preterm delivery

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The relationship between low birth weight, intrauterine growth retardation, and preterm delivery in infants born at a perinatal center is described. Betweer 20% and 30% of infants born weighing 500 to 2000 gm and nearly 50% of infants born weighing 2001 to 2750 gm had intrauterine growth retardation. For infants within the same low-birth weight group, infants with intrauterine growth retardation had one half to one sixth of the neonatal mortality rate of non-growth-retarded infants. However, only in the 501 to 1000 gm group did the difference in mortality between infants with and without intrauterine growth retardation substantially influence the composition of the group of survivors. (AM J OBSTET GYNECOL 1985; 152:980-4.)

Key words: Low birth weight, intrauterine growth retardation, preterm delivery

Nearly 50% of the infants in the 501 to 1000 gm birth weight group who were discharged live from the neonatal intensive care unit at the University of Alabama in Birmingham over the last decade have been classified as growth retarded. This study was undertaken to determine why such a high percentage of the survivors in this birth weight group were growth retarded and to better understand the relationship between low birth weight, intrauterine growth retarda-

groups within this range, the relationship between intrauterine growth retardation and preterm delivery has not been explored. Since the mortality rates and both the short- and long-term morbidity rates appear different for infants with intrauterine growth retardation and preterm new-

tion, and preterm delivery. Previous studies addressing

these relationships suggest that approximately one

third of live-born infants weighing <2500 gm are growth retarded.1 However, for specific birth weight

borns,2-5 it is likely that the frequency of these charac-

teristics in any birth weight group will influence many

of the specific outcomes that are measured. Because

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neonatal mortality and newborn follow-up studies are most frequently reported in 250 or 500 gm birth weight groupings, an understanding of the relationship between intrauterine growth retardation and preterm de-

Reprint requests: Robert L. Goldenberg, M.D., Department of Obstetrics and Gynecology, University of Alabama a: Birmingham, School of Medicine, University Station, Birmingham, AL 35294. livery for these smaller birth weight groups is important. This study was performed to examine the distribution of intrauterine growth retardation and preterm delivery within the various birth weight groups and the influence of this distribution on subsequent survival.

Material and methods

The study population consisted of all infants born live at the University of Alabama in Birmingham teaching hospitals from 1979 through 1981. These patients were predominantly of lower socioeconomic status, and 70% were black. About 8% of the births resulted from high-risk maternal-fetal transports. Data for this study were obtained in part from computerized state vital statistics records drawn from birth and death certificates6 and from the University of Alabama in Birmingham computerized obstetric data base previously described in detail.7

For this study, birth weight was recorded to the nearest 10 gm based on a measurement made within 1 hour after delivery. The best estimate of gestational age, presented as weeks completed from the last menstrual period, was derived from all available obstetric prenatal information including the last menstrual period, an early physical examination, the presence of quickening and fetal heart tones, and ultrasound examinations. Neonatal death was defined as the death before discharge from the hospital of any live-born infant weighing > 500 gm.

Neonatal mortality rates in infants with and without intrauterine growth retardation were initially evaluated in birth weight groups of 100 and 250 gm. These smaller groupings were consolidated into 500 gm birth weight groupings only after it was demonstrated that no trends apparent with use of the smaller birth weight groupings were lost by displaying data in this fashion. Specifically, we found no evidence within any of the three 500 gm, low-birth weight groups that the difference in survival could be accounted for by there being more infants with intrauterine growth retardation in the heavier part of the birth weight span.

A preterm delivery was defined as any birth that occurred before 37 completed weeks of gestation. Although a number of definitions exist for intrauterine growth retardation, we chose to use the tenth-percentile birth weight at each gestational age based on the tables of Brenner et al.8 We used these data rather than the more widely used Colorado data² because Brenner's population more closely resembled our own and because the tenth-percentile birth weight for each gestational age in that study very nearly approached the values that we have derived for the state of Alabama. The tenth-percentile measurements below which an in-

Table I. The tenth-percentile birth weight for gestational age as determined by Brenner et al.8

Gestational age (wk)	Tenth percentile birth weigh (gm)	
21	280	
22	320	
23	370	
24	420	
25	490	
26	570	
27	660	
28	770	
29	890	
30	1030	
31	1180	
32	1310	
33	1480	
34	1670	
35	1870	
36	2190	
37	2310	
38	2510	
39	2680	
40	2750	
41	2800	
42	2830	
43	2840	
44	2790	

fant is classified as having intrauterine growth retardation are shown in Table I.

Results

During the study years 12,818 live births occurred at the University of Alabama in Birmingham hospitals. In Fig. 1 these are classified as term or preterm and with or without intrauterine growth retardation. In the birth weight groups <1001 gm, all but one infant was preterm, but of these, 20% to 30% had intrauterine growth retardation. In the three birth weight groups from 1001 to 1750 gm, while nearly all newborns were preterm, there were 2% to 5% that were 37 weeks' gestational age or more. These few term infants certainly had intrauterine growth retardation, but in addition, another 15% to 25% of the preterm newborn infants in these birth weight groups were found to have intrauterine growth retardation as well. Conversely, 70% to 80% of the infants born weighing 501 to 1750 gm were found to be preterm but were above the tenthpercentile birth weight for gestational age.

The four birth weight groups from 1751 to 2750 gm each had an increasing percentage of growth-retarded infants born at term. In fact, at between 2501 to 2750 gm, which is above the traditional cutoff point for low birth weight, 49% of newborn infants were found to be below the tenth-percentile birth weight for gestational age and therefore had intrauterine growth re-

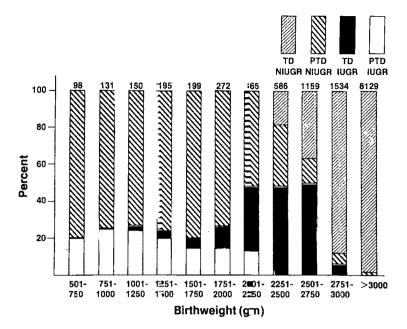


Fig. 1. The percent of infants in various birth weight categories classified as term and not growth retarded (TD NIUGR), preterm and not growth retarded (FTD NIUGR), term and growth retarded (TD IUGR), and preterm and growth retarded (PTD IUGR).

Table II. The neonatal mortality rate per 1000 line births and th∈ percent of live births and neonatal survivors by birth weight groups which had and cid not have intrauterine growth retardation

		Birtleweight (gm)					
	50.	1-1000	160	1-1500	150	01-2500	
	Intrauterine growth retardation	No intrauter:ne growth retardatior	Intrauterine growth retardation	No intrauterine growth retardation	Intrauterine growth retardation	No intrauterine growth retardation	
Neonatal mortality rate per 1000 live births	268	537	. 056	134	003	019	
Live births (%) Survivors (%)	22 31	78 69	22 24	78 76	43 44	57 56	

tardation. Since no infant born weighing >2840 gm had intrauterine growth retardation by the definition used, only a small percentage of the infants born weighing 2751 to 3000 gm and none weighing >3000 gm had intrauterine growth retardation.

Neonatal mortality rates for infants with and without intrauterine growth retardation in three low-brth weight groups are shown in Table II. In the 50 °E to 1000 gm and 1001 to 1500 gm birth weight groups, infants with intrauterine growth retardation had Ealf the neonatal mortality rate of non-growth-retarded-infants. Infants with intrauterine growth retardation born weighing 1501 to 2500 gm had one sixth of he neonatal mortality rate of infants without growth retardation. However, only in the lowest birth weight group, 501 to 1000 gm, did the difference in neonetal mortality rate between infants with and without intra-

uterine growth retardation substantially affect the percent of infants discharged alive who had intrauterine growth retardation. In this group, while only 22% of live-bo□n infants had intrauterine growth retardation, the excess mortality in non-growth retarded infants resulted n 31% of the surviving infants having intrauterine growth retardation. In the two other birth weight groups, the differential mortality between infants w th and without intrauterine growth retardation raised □he percentage of infants with intrauterine growth retardation at discharge from 22% to 24% in the 10□ to 1500 gm group and from 43% to 44% in the 15□ to 2500 gm group, respectively.

Comments

This . tudy, which explored the relationship between intrauterine growth retardation, preterm delivery, and

neonatal mortality in a population of low-birth weight infants born at a university hospital perinatal center, was undertaken as a first step in understanding various types of outcome studies presented by birth weight alone. The proportion of live-born infants classified as preterm, preterm with intrauterine growth retardation, term, and term with intrauterine growth retardation was not constant over the range of low-birth weight infants studied. As an example, from 501 to 1500 gm, nearly all live-born infants were preterm, but about 25% had intrauterine growth retardation as well. Between 2001 and 2500 gm there was a progressive decrease in the percent of live-born infants that were preterm, but nearly 50% consistently had intrauterine growth retardation. Infants with birth weights between 2501 and 2750 gm, weights which would not qualify as low birth weight by the standard definition,9 nevertheless had intrauterine growth retardation almost 50% of the time.

In our obstetric population, as the gestational age increased to term, the number of infants at each gestational age increased as well. A similar trend appears in the Alabama vital statistics data10 and in every population-based gestational age study known to us. Since intrauterine growth retardation is defined as below the tenth-percentile birth weight at each gestational age, as expected, the lower birth weight groups were found to contain numbers of infants with intrauterine growth retardation in excess of the expected 10%. Conversely, the heaviest birth weight groups, those that contain the vast majority of infants, had no infants with intrauterine growth retardation.

A higher neonatal mortality rate for infants with no intrauterine growth retardation compared to growth retarded infants was found in all low-birth weight groups. Follow-up studies of neonatal survivors, presented by birth weight group alone, therefore, appear likely to be overrepresented by infants with intrauterine growth retardation and underrepresented by infants of appropriate gestational age when compared to the group at birth. In the lowest birth weight group, 501 to 1000 gm, the difference in neonatal mortality rate between preterm infants with size appropriate for gestational age and those more advanced preterm infants with intrauterine growth retardation markedly affected the composition of the group of survivors. However, because of the relative rarity of death in infants born weighing >1000 gm, the ratio in these neonatal survivors among those with and those without intrauterine growth retardation was not found to be substantially different from that of the group at birth.

This study therefore identified two reasons why the 501 to 1000 gm group in our follow-up program had such a large percentage of infants classified as having intrauterine growth retardation. First, very low-birth

weight infants frequently have intrauterine growth retardation. Second, growth retarded infants within the same birth weight group die less frequently than do non-growth-retarded infants. We believe, but cannot prove from this data set, that in the years before 1979, ir. the 501 to 1000 gm group, infants with intrauterine growth retardation had an even greater selective survival rate over non-growth-retarded infants, thereby accounting for the nearly 50% incidence of intrauterine growth retardation in 501 to 1000 gm neonatal sur-

These findings suggest that the customary method of reporting low-birth weight outcome studies by birth weight alone is not appropriate. Clearly, in any low-Lirth weight group the gestational age distribution, and especially the proportion of infants that have intrauterine growth retardation, will very likely influence any outcome measure made. For example, in considering infants born weighing 751 to 1000 gm, a high proportion of growth-retarded infants of an advanced gestational age will likely cause mortality rates for the group as a whole to be lower than for a group of mostly non-growth-retarded, less mature infants. Similarly, a group of infants born weighing 2251 to 2500 gm with a large proportion of growth-retarded infants will likely have different long-term follow-up results than a population of appropriate-for-gestational age infants who were n the same birth weight group. Therefore, for the presentation of either mortality or follow-up studies of low-birth weight infants to be meaningful, it is apparent that in addition to the demographic characteristics and birth weight distribution, the gestational age distribution and/or the proportion of infants found to have intrauterine growth retardation within the popalation studied must be described.

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Labor and delivery in the presence of mitral stenosis: Central hemodynamic observations

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During a 1-year period, eight patients with New York Heart Association Class III or IV mitral stenosis were studied throughout the peripartum period with a pulmonary artery catheter. All patients were delivered vaginally. Intrapartum management was based upon cautious diuresis for preload optimization and heart rate control with propranolol. A mean increase in pulmonary capillary wedge pressure of 10 mm Hg was observed in the immediate postpartum period. Only two patients demonstrated a significant increase in cardiac output during this same time period. Central vencus pressure correlated poorly with pulmonary capillary wedge pressure in seven of eight patients. Neonatal outcome was uniformly excellent. With the management approach described, no patient exhibited deterioration of cardiopulmonary status during the peripartum period. (AM J OBSTET GYNECOL 1985;152:984-8.)

Key words: Mitral stenosis, cardiac disease, pulmonary artery catheter, hemodynamics

The hemodynamic alterations associated with pregnancy pose unique problems for the woman with mitral stenosis. This lesion is the most common valvular defect associated with maternal death in pregnancy. With severe disease, such women face a pregnancy-related mortality of 5%²; labor, delivery, and the immediate puerperium appear to be the times of maximal risk. This prospective study was undertaken in an effort to define more clearly the hemodynamic alterations associated with labor and delivery in patients with such rheumatic valvular lesions and to evaluate a management scheme based on aggressive intrapartum hemodynamic monitoring, cautious preload reduction, and heart rate control.

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Material and methods

During a 1-year period, eight patients with severe mitral valve disease were delivered at Los Angeles County/University of Southern California Women's Hospital. All patients had New York Heart Association Class III or IV disease and a history of rheumatic fever. In all cases, the nature of the lesion was documented by echocardiography and/or cardiac catheterization. In addition to mitral stenosis, four patients had evidence of additional valvular lesions (Table I). Cardiac rhythm was sinus in all cases. A flow-directed pulmonary artery catheter was placed either prior to elective induction (two patients) or in early spontaneous labor (six patients). During labor patients were in a special intensive care section of the labor and delivery suite. All catheters were placed by S. L. C. using the right internal jugular approach. Placement was confirmed by waveform and postplacement chest x-ray films. Hemodynamic data were collected with several monitor/recording systems (Hewlett-Packard, Physiocontrol, Datascope) and a Model 5840-A thermal dilution cardiac output computer (American Edwards Laboratories). Reported measurements were made between contractions with patients in the lateral recumbant position with evaluation of the head and chest.

Table I. Clinical data for eight patients with mitral stenosis

	² atient							
	1	2	3	4	5	6	· 7	8
Age (yr)	19	24	26	· 26	24	35	21	22
Parity	0	2	1	0	0	0	0	0
Cardiac lesion	MS, MI	MS, AI	MS	MS	MS	MS	MS, AI	MS, MI, AS. AI
Functional Class (NYHA)	III	III	IV	IV	III	IV	III	IV
Intrapartum therapy	_	_	F*	FP†	-	F*	F*	F*
Gestational age (wk)	36	40	32	39	39	40	39	37
Birth weight (gm)	2650	3450	1979	4200	2920	3405	3360	2880
Presentation	V	·v	В	V	V	V	V	V
Delivery	SVD	SVD	SVD	OF	SVD	OF	SVD	OF
Apgar scores	8, 9	9, 9	8, 9	8, 9	5, 9	8, 9	7, 8	9, 9

MS = Mitral stenosis; MI = mitral insufficiency; AI = aortic insufficiency; AS = aortic stenosis; NYHA = New York Heart Association; V = vertex; B = breech; SVD = spontaneous vaginal delivery; OF = outlet forceps.

All patients underwent continuous electronic fetal heart rate monitoring throughout labor. Statistical analysis was with a two-tailed paired t test.

Results

Clinical data for the eight patients are presented in Tables I and II. All patients had been functional Class I or II prior to pregnancy. The mean duration of labor was 11.5 hours for the six nulliparous patients and 7.0 hours for the two parous patients. All patients were delivered vaginally, seven with vertex presentations and one with a breech. Outlet forceps were applied in three cases. There were no midforceps deliveries. Epidural anesthesia was induced in the active phase of the first stage of labor in Patients 1, 6, and 8. No significant changes in hemodynamic parameters were observed with epidural activation. Peripartum hemodynamic changes are detailed in Table II and Fig. 1. A significant mean (±SD) increase in pulmonary capillary wedge pressure of 9.9 ± 6 mm Hg was observed in the immediate postpartum period (p < 0.01). The mean increase in central venous pressure (2.4 mm Hg) was not statistically significant. The central venous pressure differed from pulmonary capillary wedge pressure by 10 mm Hg or more at some time during labor and delivery in seven of eight patients. Infant outcome was uniformly good (Table I); there was no evidence of fetal distress during labor and there were no 5-minute Apgar scores <8. No fetus was below the tenth percentile in weight when analyzed according to the standard growth curve for California live births; however, all but the single macrosomic infant (Patient 2) fell below the fiftieth percentile for gestational age.

In five cases, intrapartum preload reduction was felt to be clinically indicated and was accomplished with

Table II. Peripartum hemodynamic indices for patients with mitral stenosis*

	Mean ± SD
First stage of labor	
MAP (mm Hg)	89.3 ± 10.7
P (bpm)	84.7 ± 10.6
CVP (mm Hg)	10.0 ± 4.3
MPA⊇ (mm Hg)	30.6 ± 16.3
PCWP (mm Hg)	21.4 ± 11.0
CI (L/min/m²)	4.6 ± 1.8
Second stage of labor,	
15-30 min before delivery	
MAF (mm Hg)	89.3 ± 7.6
P (bgm)	89.8 ± 19.4
CVP (mm Hg)	5.9 ± 5.0
MPAP (mm Hg)	23.1 ± 13.1
PCWP (mm Hg)	14.1 ± 10.8
CI (L/min/m²)	4.3 ± 1.5
5-15 min post partum	
MAF (mm Hg)	84.1 ± 9.0
P (bpm)	95.9 ± 17.5
CVP (mm Hg)	8.3 ± 5.4
MPAP (mm Hg)	33.9 ± 17.1
PCWP (mm Hg)	24.0 ± 10.4
CI (L/min/m²)	4.3 ± 1.7
18-24 hr post partum	
MAF (mm Hg)	83.0 ± 10.6
P (bpm)	81.3 ± 13.6
CVP (mm Hg)	6.5 ± 3.6
MPAP (mm Hg)	19.0 ± 11.8
PCWP (mm Hg)	11.1 ± 7.0
CI (_/min/m²)	3.6 ± 1.2

*MAP = Mean arterial pressure; P = pulse; CVP = central venous pressure; MPAP = mean pulmonary artery pressure; PCWP = pulmonary capillary wedge pressure; CI = cardiac index. Individual values available upon request.

intrarenous furosamide. In the remaining three cases, strict fluid restriction (50 to 60 ml/hr) alone was instituted. Additional intrapartum pharmacologic manipulation was carried out in one case (Patient 4) in which

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^{*}Furosemide.

[†]Furosemide/propranolol.

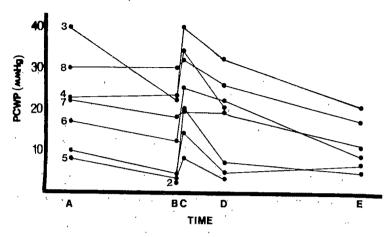


Fig. 1. Intrapartum alterations in pulmonary capillary wedge pressure (PCWP) in eight patients with mitral stenosis. A, First-stage labor. B, Second-stage labor, 15 to 30 minutes before delivery. C, 5 to 15 minutes post partum. D, 4 to 6 hours post partum. E, 18 to 24 hours post partum.

maternal tachycardia was associated with a fall in blood pressure to 74/40 mm Hg. Cautious intravenous administration of 1 mg of propranolol resulted in a fall in heart rate with restoration of cardiac output and blood pressure.

Two patients (Nos. 3 and 8) had clinical evidence of pulmonary edema upon admission. One patient improved and the other remained stable during labor, delivery, and the postpartum period. No patient without clinical pulmonary edema at admission developed it during the entire peripartum period. One patient (No. 3) with severe secondary pulmonary hypertension had an uncomplicated delivery and immediate postpartum course and was discharged after being evaluated for valve replacement. She was readmitted on postpartum day 31 to the medical intensive care unit in intractable congestive heart failure and died 5 days later. There was no other significant morbidity or mortality and no catheter-related complications were observed.

Comment

The pregnant woman experiences significant hemodynamic alterations during the antepartum and intrapartum periods. During the course of normal gestation, blood volume increases 40% to 50% and is accompanied by an increase in heart rate of 18% and in cardiac output of 50%. Labor and delivery present an additional burden upon the maternal cardiovascular system. Hansen and Ueland¹ have shown an increase in cardiac output in normal pregnancy of up to 45% during the late second stage of labor, with an additional 15% increase during contractions. Such changes seem to be largely on the basis of sympathetic-mediated increases in heart rate, as well as increased stroke volume during contractions. In addition, the normal pregnant

patient may experience an increase in cardiac output of up to 65% during the immediate postpartum period.5 This is felt to be due to postpartum volume shifts, including release of vena caval obstruction by the pregnant uterus and the decreased vascular capacitance associated with the loss of fetus and placenta and subsequent uterine contraction. Such preload changes are readily accommodated by increased cardiac output in the patient with a normal heart. However, the patient with a relatively fixed cardiac output (for instance, mitral stenosis) may be unable to accommodate such fluctuations in preload, leading to increased wedge pressure and hydrostatic pulmonary edema. For reasons mentioned previously, the immediate postpartum period appears to be especially hazardous in this regard.6 The postpartum decrease in plasma colloid osmotic pressure that accompanies normal pregnancy would also contribute to the development of pulmonary edema under these circumstances. Only two patients in the current series demonstrated a significant postpartum increase in cardiac output. Increases of 17% and 39% were seen in Patients 2 and 5 within 10 minutes after delivery. As might be expected, the increase in pulmonary capillary wedge pressure in these patients was less than the mean. Such an ability to increase cardiac output, albeit to a limited extent, presumably reflects less severe disease. All other patients failed to exhibit a postpartum increase in cardiac output and appear to have had maximum cardiac output prior to delivery. It was in this group of patients that the greatest increases in postpartum wedge pressure were seen.

Patients in whom mitral disease has led to the development of secondary pulmonary hypertension are additionally vulnerable to decreases in right heart preload. Such changes adversely affect already compromised pulmonary perfusion and may be rapidly fatal.

In obstetrics, such problems are often secondary to hemorrhage or conduction anesthesia-related hypotension. Indeed, while the pregnancy-related mortality associated with severe mitral stenosis is only 5%, a 30% to 50% mortality is reported in patients with pulmonary hypertension of any etiology.8.9

In the patient with mitral stenosis, intrapartum fluctuations in cardiac output may be minimized with the use of epidural anesthesia. In certain cases, intrapartum preload reduction, in anticipation of postpartum volume shifts, may also help avoid postpartum pulmonary edema. However, peripartum hemodynamics of patients with mitral stenosis have not previously been documented. In addition, the fetal effects of maternal preload manipulation have not been addressed. Our results indicate that in patients with Class III or IV mitral disease, a mean postpartum increase in pulmonary capillary wedge pressure of 10 mm Hg may be anticipated. However, the range was wide (0 to 18 mm Hg). This increase was greatest in those patients who entered labor with the highest wedge pressures (Fig. 1). The fact that this increase was inconsistent and not seen in two patients may reflect the intrapartum preload reduction in those patients. A pulmonary capillary wedge pressure of 20 to 22 mm Hg is associated with the onset of radiographically evident pulmonary congestion,10 frank pulmonary edema being observed as the pulmonary capillary wedge pressure approaches 28 to 30 mm Hg. Thus a patient with a predelivery pulmonary capillary wedge pressure of ≤14 mm Hg experiencing the observed mean or mean + 1 SD postpartum rise in pulmonary capillary wedge pressure would not be expected to develop clinically significant postpartum pulmonary edema. While further reduction in antepartum preload may seem even more desirable, several considerations would seem to militate against such therapy. In patients with mitral stenosis, pulmonary capillary wedge pressure is not an accurate measurement of left ventricular preload. Patients with such valvular lesions may be dependent upon high normal or elevated left atrial pressures to maintain adequate ventricular filling and cardiac output. Thus any preload reduction must be undertaken cautiously, with constant attention to cardiac output and blood pressure. Active diuresis is not always necessary in patients who enter labor with evidence of only mild fluid overload. In such patients, simple fluid restriction and the associated sensible and insensible fluid losses that accompany labor may result in a significant fall in wedge pressure prior to delivery (Patients 1 and 5).

An equally important hemodynamic consideration in patients with mitral stenosis is heart rate control. Tachycardia of any etiology (including labor, pain, or anxiety) leads to shortened diastole and ventricular filling time. Since patients with mitral stenosis are highly dependent upon adequate diastolic filling time, such tachycardia may result in a fall in cardiac output and hypotension. Routine oral propranolol therapy during the stress of labor may be a consideration; however, we found its use unnecessary in seven of eight patients in this series.

Previous recommendations for delivery in patients with cardiac disease have included the liberal use of midforceps to shorten the second stage of labor. In cases of severe disease, cesarean section with the use of general anesthesia has been recommended as the mode of delivery least likely to result in hemodynamic compromise.2. 11. 12 Where intensive monitoring of the intrapartum cardiac patient cannot be carried out in the manner described here, this recommendation for elective cesarean section may be valid. However, with the aggressive management scheme presented, our experience suggests that vaginal delivery is safe even in patients with severe disease and pulmonary hypertension. Additionally, we found it unnecessary to resort to potentially traumatic midforceps deliveries. Labor and delivery, as well as cautious intrapartum diuresis, were tolerated well by the fetuses in this study.

A growing body of evidence indicates that assessment of central venous pressure is a poor and potentially misleading indicator of left heart preload in a number of clinical situations. 13-15 We found central venous pressure to differ from pulmonary capillary wedge pressure by at least 10 mm Hg at some time during labor and delivery in seven of eight patients. In such cases, clinical reliance upon assessment of central venous pressure alone would have been misleading. In addition, while the postpartum rise in pulmonary capillary wedge pressure was highly significant, no similar rise in central venous pressure was observed.

Finally, the propensity for late postpartum death (as in Patient 3) in patients with pulmonary hypertension has been noted previously.16 This may be related to a "rebound" worsening of pulmonary hypertension secondary to changing hormonal balance in the postpartum period. It remains to be seen if prolonged hospitalization of these patients would prevent such deaths.

These observations suggest the following guidelines for the intrapartum management of patients with Class III or IV mitral stenosis:

- 1. Oxygen administration and labor in the lateral recumbent position are essential.
- 2. A pulmonary artery catheter should be placed prior to induction or in early labor to guide hemodynamic management. A central venous pressure line alone is inadequate.
- 3. Fluid restriction is essential, and cautious intrapartum reduction of pulmonary capillary wedge pressure to the 14 mm Hg range is a desirable goal. This

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must be done with careful monitoring of cardiac output and blood pressure. Further reductions in preload are unnecessary and may be harmful to mother or fetus.

- 4. When tachycardia is marked or associated with falling cardiac output and blood pressure, cautious intravenous administration of propranolol may be valuable. Prophylactic administration of oral propanolol in early labor may also be a consideration for any patient in whom the pulse is >100 bpm.
- 5. Epidural anesthesia is indicated in the active phase of labor. Continuous activation during the immediate postpartum period may also reduce venous return via peripheral vasodilatation and help avoid postpartum pulmonary edema.
- 6. When intensive intrapartum monitoring is available, cesarean section should be reserved for obstetric indications only.

In patients with primary valvular disease that is complicated by secondary pulmonary hypertension, the above recommendations may not apply. Since systemic hypotension or any fall in right heart preload may be rapidly fatal, the maintenance of adequate preload must be a primary consideration, even at the expense of incurring pulmonary edema. In general, conduction anesthesia, with its potential for hypotension, should be avoided in such patients.

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Practice patterns and attitudes toward education among Canadian obstetricians and gynecologists

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A group of 1370 specialists in obstetrics and gynecology were surveyed for information about practice patterns, continuing medical education preferences, and their perception of the adequacy of their own residency training. The overall response rate was 65.7%. More than half were in solo practice, practiced in communities of over 250,000, had been in practice for more than 10 years, or had a full or part-time appointment with a Canadian medical school. A wide range of continuing medical education methods were used. Journals were ranked highest by 41%. It is disturbing that very few physicians (15%) indicated any involvement in practice audit. The quality of residency training was ranked low in a number of areas including genetic counseling, ultrasound, neonatology, intensive care, colposcopy, sexual dysfunction, marital counseling, and hysteroscopy. The survey highlights a number of areas that merit the attention of Canadian programs in postgraduate and continuing medical education in obstetrics and gynecology. (AM J OBSTET GYNECOL 1985;152:989-94.)

Key words: Practice patterns, continuing education, training

This study was developed as a collaborative project of the Association of Professors of Obstetrics and Gynaecology of Canada and the Society of Obstetricians and Gynaecologists of Canada. University departments of obstetrics and gynecology and the Society wanted to assess the current nature of the practice of obstetrics and gynecology in Canada, the preferences of consumers of specialty continuing medical education, and qualitative and quantitative aspects of residency training viewed from the perspective of experience in clinical practice. To some extent this survey provides feedback to those involved in providing postgraduate education and continuing medical education in obstetrics and gynecology in Canada.

Methods

A survey questionnaire form was designed following the format developed by Feldman et al. The six-page, self-administered questionnaire solicited demographic information, practice patterns, continuing medical education patterns and preferences, and perceptions of the adequacy of residency training in obstetrics and gynecology. A professional marketing survey company was employed to mail the questionnaires to all obste-

tricians and gynecologists in Canada. The questionnaire was printed in both English and French and mailec according to the language preference of the physician as recorded in the mailing lists used. Each questionnaire was accompanied by a covering letter from the presidents of the two sponsoring organizations the Association of Professors of Obstetrics and Gynaecology of Canada and the Society of Obstetricians and Gynaecologists of Canada) urging their members to respond to the questionnaire.

Of 1370 questionnaires mailed, 597 were received back within 4 weeks. Nine weeks later, a second mailing was sent to nonresponders; in this mailing 304 completed questionnaires were received, for a total of 901 respondents. The overall response rate was 65.7%. No attempt was made to exclude from analysis respondents or nonrespondents who were identified as inappropriate to the survey (e.g., retired, deceased, not in practice, or noncertified), since such information was not uniformly available for all addressees. The completed questionnaires were keypunched and subjected to computer analysis.

Results

There was a fairly even distribution of respondents over the range of age groups (Table I). Year of graduation from medical school also showed an even distribution. Responses were received from physicians in all 10 provinces and the territories. Ontario and Quebec obstetricians and gynecologists made up 39% and 24% respectively of the sample. More than half of the respondents practice in large communities of a quarter

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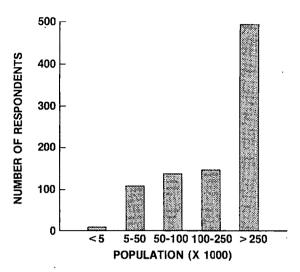


Fig. 1. Size of community in which Canadian obstetricians and gynecologists practice.

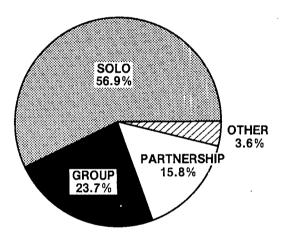


Fig. 2. Type of practice of Canadian obstetricians and gynecologists.

of a million people or more (Fig. 1). The average respondent has been in practice for 15 years and about one third of respondents for <10 years.

More than half of the respondents are in solo practice (Fig. 2). Of those over 40 years of age, 67% are in solo practice and 31% in group practice or partnership; of those under 40 years of age, 57% are in group or partnership practice and 39% in solo practice. Full-time faculty members were more often in group practice or partnership, but 43% indicated they were engaged in solo practice (compared with 65% for practitioners without university affiliation). The type of practice appeared to have no significant bearing on continuing medical education preferences.

More than half of the survey respondents had a fullor part-time appointment with a medical school. Since from another source (Association of Professors of Obstetrics and Gynaecology of Canada Faculty Registry) we know that at the time of the survey there were 598

Table I. Age distribution

Age range (yr)*	n	%
26-30	22	2
31-35	122	14
36-40	144	16
41-45	143	16
46-50	126	14
51-55	128	14
56+	212	24

^{*}Mean age, 46.6 years (missing data in 4 responses).

Table II. Special areas of interest in obstetrics and gynecology

Subject*	n	%
Endocrine/infertility	348	40
General obstetrics and gynecology	323	37
Maternal/fetal medicine	301	34
Family planning	204	23
Oncology	160	18
Adolescent gynecology	104	12
Sexual counseling	67	8
Urodynamics	64	7
Psychosomatic medicine	44	5
Other	77	9

^{*}Some multiple responses (missing data in 21 responses).

obstetricians and gynecologists with faculty appointments in Canadian medical schools, we can estimate an 82% response rate for those with full- or part-time appointments and a 52% response rate for those without a university appointment. This indicates a bias in the responses to this survey, since those with university appointments are more likely to have ready access to various types of continuing medical education and are also directly involved in current postgraduate training. However, it should be noted that, despite a lower response rate, the "no appointment" group is still the largest single subgroup responding to the survey (40%).

A majority of respondents indicated that they had special areas of interest in obstetrics and gynecology (Table II). These data refer to areas of interest but not necessarily to subspecialty, since no formal subspecialty qualification existed in obstetrics and gynecology in Canada at the time of the survey. More than one third expressed interest in two of the three subspecialty areas: endocrine/infertility, maternal/fetal medicine, and gynecologic oncology. Fewer than 10% expressed a special interest in sexual counseling, urodynamics, or psychosomatic medicine. The response rate was high to the question on areas of interest (880 of 901), indicating that most respondents have an interest in at least one special area of the discipline.

Respondents were asked to rank the forms of con-

Table III. Methods of continuing medical education rated as highly useful

Method	n	%	
Journals	701	78	
Professional meetings	552	61	
Workshops/refresher courses	468	52	
Rounds	435	48	
Personal communication	252	28	
Tapes	242	27	
Other methods	468	52	

Table IV. Form of continuing medical education which most recently caused change in clinical practice

Туре	n	%	
Journals	366	40.6	
Meetings	347	38.5	
Rounds	302	33.5	
Workshops/courses	238	26.4	
Informal discussions	213	23.0	
Tapes	42	4.7	
Other/not stated	36	4.0	

tinuing medical education listed in Table III to indicate those most useful for their learning. Those methods ranked 1, 2, or 3 were considered highly useful for purposes of analysis. Journals, professional meetings, workshops/refresher courses, and rounds were the most preferred forms of continuing medical education. Age, geographic location, or size of community did not significantly influence the selection of journals as the preferred method of continuing medical education.

Overall, the reading of journals was reported to be the continuing medical education activity most likely to motivate a change in patient management (Table IV). Attendance at rounds and meetings was also viewed as valuable; personal communication was considered to be useful by <20% of respondents. Whereas geographic location in Canada had a modest influence on the type of activity reported, the size of the community had a striking influence. Respondents in communities of >250,000 people reported a much greater preference for rounds and meetings compared with their colleagues in smaller communities, who reported journals as the type of continuing medical education activity most likely to motivate change in patient management. Full-time and part-time faculty members attributed over twice as great an influence on change to rounds as did those obstetricians and gynecologists with no university affiliation. Reliance on meetings and personal communication was similar in these groups.

Physicians were asked about their involvement in research (Table V). A very large difference in the re-

Table V. Type of research projects physicians engage in

Type*	n	%	
Laboratory	52	6	
Descriptive	98	11 15 18	
Audit	136		
Trials	164		
Epidemiology	58	6	
Educational	50	6	
Other	16	2	
None/not stated	561	62	

^{*}Some multiple responses.

Table VI. Scores for residency training in clinical obstetrics*

	% of low scores		
	Quantity	Quality	
Operative obstetrics	7	6	
Postpartum care	9	10	
Prenatal care	11	9	
Identification of high-risk patients	15	11.	
Management of high risk patients	15	11	
Medical complications	16	12	
Fetal assessment	20	14	
Resuscitation of newborn infants	42	37	
Neonatology	60	52	
Ultrasound diagnosis	62	50	
Genetic counseling	74	61	

^{*}It is clear that the responses indicate strong disapproval of the quantity and quality of training in Neonatology, Obstetric ultrasound, and genetic counseling.

sponse rates to this question can be noted between the full-time faculty members (84%) and the group of parttime faculty members and those with no university affiliation (32%). The question may have been unclear because the categories used were of necessity somewhat arbitrary.

Eighty-three percent of those surveyed responded to the question about the duration of residency training. Two thirds felt that the current length of training was appropriate. This was not influenced by age, sex, or association with a medical school. However, francophone respondents recommended five years of training more often (26%) than did anglophone respondents (12%). Overall, 16% of respondents felt that the training period should be 5 years, and 5% felt 3 years of training to be preferable.

Respondents were asked to rate on two separate fivepoint scales the quality and quantity of their residency training in a number of subject areas of obstetrics and gynecology from the perspective of their current practice (Table VI and VII). In these tables, low scores indicate that respondents assigned a score of 1 or 2 to the item (i.e., too little in quantity or poor in quality).

Table VII. Scores for residency training in clinical gynecology*

,	% of low sc-es		
	Quantity	Crality	
Operative gynecology	10		
Pathology	21	17	
Urinary problems	22	22	
Contraception	21	.8	
Ambulatory gynecology	28	20	
Infertility diagnosis and treatment	26	_4	
Laparoscopy	25	_9	
Gynecologic endocrinology	38	52	
Surgical oncology	39	를 .1	
Medical oncology	43	Ξ 6	
Bowel surgery	48	Ξ7	
Intensive care	51	≡2	
Ultrasound diagnosis	68	=4	
Colposcopy	70	=3	
Sexual dysfunction	84	77	
Marital counseling	89	34	
Hysteroscopy	87	₹9	

^{*}More than half of respondents assigned low scores their training in intensive care, ultrasound diagnosis, colpccopy, sexual dysfunction, marital counseling, or hysteroscop-

Response rates to different items varied, reflecting the fact that some of the items were directed to ward newer aspects of specialty practice (e.g., colposopy) which were not available when older physicians trained. Also there was a consistent similarity in patterns of response to quantity and quality scores, suggesting that these scores were not independent of each other. Dverall, the "amount of training" items were given low scores in 36.8% of responses and "quality of training" in 30.3%. Specific responses to individual items should be viewed in the light of these overall responses. We suggest that items for which >40% of scores were low represent areas that should be assessed by those responsible for residency training programs.

Table VIII outlines the responses received to the question "What changes would you like to see in Canadian residency programs in obstetrics and gyrecology?" Respondents were directed specifically to address format, content, and methods of education. The most common responses indicated a preference for more supervision, more responsibility, and more forms instruction during residency training.

Comment

Although the demographic data and practice patterns of Canadian obstetricians and gynecologist are of interest per se, they have particular relevanæ to attitudes toward and availability of continuing medical education.

The small number of women among the respondents reflects the small number of practicing obstetricans who are women. In 1982 7.2% of obstetricians and

Table VIII. Preferred methods of residency education in obstetrics and gynecology

Response	Total	% of respondents		
No change	41	7		
More supervision	204	36		
More exposure to patients	30	5		
More formal training/teaching of obstetrics and gynecology	102	18		
More resident responsibility	144	26		
Less formal training	31	6		
More seminar/workshop/self- learning type of teaching	44	8		
6-month training in obstetrics for general practitioner	37	7		
More surgery (supervised)	44	7		
More research	5	1		
More office management training	4	1		
Other comments	31	6		
Not answered	339			

gynecologists in Ontario were women,² and 10% of specialists certified in obstetrics and gynecology in Canada between 1970 and 1979 were women.³ However, this is likely to change, since 36% of obstetrics and gynecology residents in Canadian residency programs in 1982 to 83 were women,⁴ including 44% of first-year residents. This significant increase in the number of female practitioners may change the nature of the specialty over the next decade.⁵

Because of the clustering of obstetricians and gynecologists in large urban areas, many of which have a medical school, opportunities to select from various forms of continuing medical education activities are probably available to most obstetricians and gynecologists in Canada. This clustering may also explain the relatively large percentage of obstetricians affiliated with a medical school (19% full-time, 36% part-time faculty members). By contrast, a study of United States medical schools⁶ based on a 1977 questionnaire revealed 10% of Board-certified obstetricians and gynecologists were full-time faculty members.

The organization of practice is of interest. Solo practice is the predominant mode (57%) of Canadian obstetricians and gynecologists. This compares to only 40% of Canadian pediatricians in the study of Feldman et al. This tendency for younger obstetricians to engage in group practice or partnership was also noted in the American study by Mendenhall et al. They demonstrated a decreasing trend to solo practice among younger obstetricians and gynecologists; only 6.2% of those under 35 years of age reported solo practice in contrast to 43.8% of those between 55 and 64 years of age. In the present study the size of community in which the practice was located did not influence the organization of practice. Despite the relatively modest percentage of obstetricians and gynecologists engaged

in collaborative practice, responses to questions about on-call hours suggest that most are involved in some type of on-call coverage arrangement.

The response to the question about involvement in research should be interpreted with caution. However, the fact that only 15% of respondents indicated involvement in clinical audit (10% in the part-time university group) suggests that medical schools and professional organizations may have an important role to play in the future in the promotion of practice audit. The relatively large number of respondents who indicated involvement in clinical trials is surprising but may in part reflect involvement in large multicenter clinical trials.

Respondents to this survey were asked to rank the forms of continuing medical education which they felt were most useful for their learning. Curry and Putnam,8 in their study of Canadian physicians practicing in the Maritimes, noted differences between the methods physicians actually use and those they would use if they had unlimited time and funds. Individual learning methods, especially reading, predominated in the former instance, whereas in the latter, more courses, clinical traineeships, and university-sponsored local hospital programs were preferred. In a study of continuing medical education in Ontario in 1979, Davis et al.4 reported with reference to informal continuing medical education activity that the reading of journals and texts, informal discussion, and attendance at rounds were reported as activities by over 70% of respondents. Whereas reading of journals was reported nearly universally, hospital-based specialists made more frequent use of rounds and informal consultation than did their community-based colleagues. In terms of formal activity, hospital-based specialists relied more on medical school continuing medical education and scientific sessions of societies than did community-based specialists and family physicians who relied more on hospital staff continuing medical education, visiting clinician programs, and clinical traineeships. In Feldman et al.'s nationwide survey of Canadian pediatricians1 journals were reported as the preferred form of continuing medical education, with hospital rounds and meetings a distant second and third choice.

Change in physician performance and a resultant improvement in patient care are the ultimate goals of continuing medical education. The transmission of information does not of course ensure either change in physician performance or improvement in patient outcome. In fact, there is evidence that insufficient knowledge plays only a very modest role in deficient patient care. 10 The process of change in physician practice behavior is complex. Learning methods vary in their effectiveness with respect to change in diagnosis, management, prescription, and patient relationships. General practitioners and specialists studied by Geertsma et al.11 considered communication with selected local colleagues and the reading of professional journals to be the most useful authoritative sources of information on which to base change. Just how effective the various forms of continuing medical education are in increasing physician capacity (knowledge and skills), improving performance (diagnosis, management, prescribing habits), and improving patient outcomes (reduced mortality and morbidity, increased health and satisfaction) remains controversial.12

In a recent study of a small number of internists, surgeons, and gynecologists attending a continuing medical education event, it was learned that changes in practice tended to be confined to refinement of techniques (prescribing, investigation, or technical skill) rather than changes that radically altered morbidity or mortality outcomes. The precipitating factor that convinced the physician that the change should be introduced was most often the anticipated benefit in patient care and, to some degree, dissatisfaction with current practice.13

In the study under discussion here, Canadian obstetricians indicated that journals are the form of continuing medical education most likely to change their clinical practice. Therefore, in undergraduate, residency, and continuing medical education programs, we suggest that more emphasis should be placed on the critical analysis of scientific and clinical literature in order to better prepare physicians to assess published reports and their relevance to their own practice. Also it may be prudent for continuing medical education groups and professional societies to do more research into the reading preferences of physicians, since individualized continuing medical education programs are becoming more common.14

Respondents were asked to rate areas of their training in light of their practice experience. These data (Tables VI and VII) are of particular interest to those responsible for the organization and conduct of postgraduate medical education in obstetrics and gynecology in Canada. It is clear that there is strong disapproval cf the quantity and quality of training in neonatology, obstetric ultrasonography, and genetic counseling. More than half of the respondents assigned low scores to their training in intensive care, the diagnostic use of ultrasonography, colposcopy, hysteroscopy, sexual dysfunction, and marital counseling. Some of these deficiencies in training may well reflect newer developments in the specialty which were not practiced when many obstetricians and gynecologists trained.

In light of the number of areas in clinical obstetrics

and gynecology in which respondents felt inadequately prepared by their residency training (Tables VI and VII), it will be important for providers of continuing medical education to offer those forms that obstetricians and gynecologists find most useful in learning new techniques and skills as opposed to new information concerning diagnosis and management. In a study of the learning preferences of Canadian Maritime specialists, reading was ranked most important for knowledge acquisition, followed by refresher courses. For skills acquisition, refresher courses were ranked highest, followed by reading, clinical traineeships, and informal demonstrations. ¹⁴

Eskew and Watt¹⁵ reported on a survey of all residents in obstetrics and gynecology in the United States; this study had a response rate of 34%. Respondents answered that the quantity of their training was excellent to good in all areas with the exception of endocrinology, infertility, outpatient surgery, human sexuality, and office management. The most deficient areas were the last two. Instruction in and supervision of gynecologic surgery were rated excellent or good by nearly 95% of respondents. However, midforceps delivery, colposcopy, cryosurgery, and ultrasonography were not taught in 30% to 40% of the programs, and female urology was not taught in over 50%. Supervision in specialty areas was considered adequate, except in those areas in which patient volume was low. In the present study there was a consistent association between low scores for quality of training and quantity of training, which suggests that the most important need may be for adequate experience in these special subject areas.

Dunn, ¹⁶ in a well-reasoned statement, discussed the issues surrounding the appropriateness of training obstetricians and gynecologists in intestinal and urologic surgery. He concluded that the considerable difficulty in providing adequate training for all residents is of questionable value when there is little evidence for the later practical application of these skills.

The responses about preferred methods of residency training in obstetrics and gynecology indicate that a structured, organized program with adequate supervision, resident responsibility, and more formal instruction is highly desirable. Although superficially, it may appear that increased supervision and resident responsibility are incompatible, we believe that this is not so. Adequate supervision of trainees by their clinical teachers is important to ensure both a sound educational experience and a consistently high standard of patient care. Periods of time set aside for structured, formal

instruction should be an integral part of all contemporary residency training programs. Learning by design rather than by chance maximizes the efficiency of student learning and ensures the coverage of major and newer subject areas in the discipline. Critical reading and appraisal of relevant literature during residency training should be strongly encouraged, since this appears to be the primary method of continuing education for the practicing physician.

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The effects of low-dose oral contraceptives on coagulation and fibrinolysis in two high-risk populations: Young female smokers and older premenopausal women

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A study was undertaken to determine the effect of a low-dose oral contraceptive on the coagulation and inhibitory system of coagulation in 22 young healthy women who smoke and ir 15 nonsmoking healthy women between the ages of 34 and 41. Smokers showed statistically significant oral contraceptive–related procoagulant alterations in prothrombin time, thrombin time, and fibrinogen ant gen. Antithrombin III antigen and activity were significantly reduced, whereas plasminogen antigen and activity were increased. Inhibitor and fibrinolytic activity was either unaffected or enhanced by oral contraceptives in women over the age of 34: antithrombin III activity was unchanged, plasminogen antigen and activity increased (p < 0.0007), and α_2 -antiplasmin was significantly reduced (p < 0.07). Whereas usage of oral contraceptives in young smokers may initiate biochemical changes in favor of hrombogenesis, their usage in nonsmoking older women enhanced fibrinolysis and had a neutral effect on nhibition and a minimal procoagulant effect. (Am J Obstet Gynecol 1985;152:995-1000.)

Key words: Smoking, premenopausal, oral contraception, coagulation, fibrinolysis

Oral contraception usage has been reported to be associated with an increased risk of thromboembolic events,1 myocardial infarction,2 and other cardiovascular events, including subarachnoid hemorrhage and stroke.3 This risk is especially true for women over the age of 35 and in those who smoke.2 In one study the risk that a woman between the ages of 40 and 44 would require hospitalization for myocardial infarction was found to be five times greater in those using oral contraceptives than in age-matched controls not on contraceptives.2 Smoking and oral contraceptive use seem to act synergistically in increasing the risk of subarachnoid hemorrhage, stroke, and myocardial infarction. Pettiti et al.3 reported an increase in these events from a control incidence of 8.3/100,000 per year to 127.3/ 100,000 per year in patients having both risk factors. This risk is further compounded when age is super-

Numerous investigators have attempted to explain the association between oral contraceptive use and thrombosis by studying the effect of the various sex steroids used in these preparations on factors involved in coagulation and fibrinolysis. Although the majority of reports link measurable changes in some of the procoagulant and/or inhibitory systems, there is increasing doubt as to the interpretation and clinical meaning of these alterations. No published studies have demonstrated a direct cause-and-effect relationship between a measured prothrombotic change in the coagulation cascade and a clinically proven thrombus. Many of the reported changes, although perhaps statistically significant, are still well within accepted physiologic levels.

More recently the risk of induced thrombosis has been found to be significantly less in women prescribed low-dose oral contraceptives,5 whereas many of the hemostatic tests in earlier studies with high-dose preparations have also been shown to be much less affected.⁵⁻⁷ Having demonstrated that a low-dose oral contraceptive containing 35 µg of ethinyl estradiol and 0.4 mg of norethindrone (Ovcon-35) had no significant observable effects on the hemostatic mechanism in young healthy Caucasian women, this study was undertaken to evaluate the effect of the same preparation on coagulation and fibrinolysis in two subgroups perhaps at higher-than-usual risk for thromboembolism: young women who smoke and nonsmoking women over the age of 34. This was deemed necessary because of the arge number of young women who use both oral contraceptives and tobacco products and because of the need for effective contraception in women dur-

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Table I. Characteristics of patients studied

	No of patients	Age (yr)		Height =m)		Weight (kg)		No. of cigarettes smoked		Blood pressure (mm Hg)	
		Mean	Range	Mean	₹ange	Mean	Range	Mean	Range	Systolic	Diastolic
Group 1: smokers	22	21	18-24	165.9	15∈2-174.6	59.6	46.8-94.5	25	10-40	105 ± 8.0	69 ± 5.0
Group 2: over 34	15	37	34-41	165.8	157 5-172.7	67.6	53.6-81.8		_	106 ± 9.8	72 ± 8.9

Table II. Dynamic tests of coagulation (mean \equiv SEM)

	.Group ,	A: baseline- value	"B: 1-month therapy	C: 6-months therapy	D: 1-month after therapy	Statistical significance at time intervals
Prothrombin time (normal: 9.5-12.0 sec) Activated partial thromboplastin time (normal: \$35 sec)	Over 34	11.1 ± 0.5 27.4 ± 0.7	$\begin{array}{c} 11.0 \pm 0.3 \\ 10.1 \pm 0.3 \\ 26.0 \pm 0.7 \\ 25.2 \pm 1.8 \end{array}$	11.0 ± 0.3 9.9 ± 0.2 25.8 ± 0.8 25.3 ± 1.6	11.7 ± 0.2 10.9 ± 0.2 27.2 ± 0.9 24.2 ± 0.6	B, C vs. A, D: p = 0.009 B, C vs. A, D: p = 0.009 NS NS
Thrombin time (normal: 10-25 sec) Fibrinogen antigen (normal: 200-450 mg/dl)	Smokers Over 34 Smokers Over 34	13.7 ± 0.8 266.4 ± 14.5	12.6 ± 0.6 14.9 ± 0.7 316.3 ± 13.6 308.5 ± 21.2	11.7 ± 0.7 15.4 ± 0.9 316.1 ± 17.6 319 ± 19.5		B, C vs. D: p = 0.03 B, C vs. D: p = 0.03 B, C vs. D: p = 0.004 NS

ing their early climacteric years—especially these between the ages of 35 and 45.

Material and methods

The study was conducted on outpatient, healtay ambulatory women at the Center for Climacteric Sudies, University of Florida, following approval of the Institutional Review Board. Women who had no risk Tactors for oral contraceptive usage other than their smoking habit were admitted into the study (group 1). All of the participants had previously been regular oral contraceptive users. They were moderate to heavy smokers, with a mean consumption of 25 ± 15 cigarettes smoked daily (Table I). Twenty-two subjects completed the 7month study. Group 2 comprised 15 nonsmoking women over the age of 34. They were also curren_users of oral contraceptives and had no demonstrable #t-risk factors other than their age (Table I). Smokers over the age of 34 were not studied. Steroid contraception was discontinued for at least 1 month before the stury for all subjects. After informed consent was obtainec each subject had a detailed physical and gynecologic examination. The subjects were given a 6-month supply of oral contraceptives (Ovcon-35) and were see at 1 month and 6 months after their initial evaluation. A final physical examination was performed 1 morth after completion of their treatment schedule. The participants used barrier contraceptives between their sixth- and seventh-month visits. This design allowed each patient to serve as her own control. At each asit baseline (no treatment), 1 month, 6 months, and 7 months (no treatment)—venous samples of blooc were

obtained at 9:00 AM after a 10-hour overnight fast; 9 ml cc of whole blood was collected in tubes containing 1 cc of 3.8% sodium citrate and centrifuged immediately at 4500 rpm for 5 to 10 minutes. Plasma was separated and kept frozen at -70° C until analysis. All samples were drawn between the fifteenth and twenty-first day of the menstrual cycle. No woman experienced thrombophlebitis or related vascular problems.

Tests representative of both coagulation and its inhibition were performed. The prothrombin time, activated partial thromboplastin time, and thrombin time were measured with use of a fibrometer and standard laboratory techniques. Clottable fibrinogen was determined by the Claus method.8 Fibrinogen antigen, plasminogen antigen, a2-macroglobulin antigen, and antithrombin III antigen were determined by radial immunodiffusion with use of plates purchased from Calbiochem-Behring (m-Partigen TM). Plasminogen activity, antithrombin III activity, and α2-antiplasmin activity were determined by fluorometric assays (Protopath, American Dade, Miami, Florida). The variation for Protopath for antithrombin III is 7% and for plasminogen 5%. All tests were performed by a technician (D. L.) blinded to the group assignments. Results were compared to the baseline for each patient and also to levels deemed by this laboratory to be normal based on local determination of pooled normal plasma. These levels are comparable to those in the literature.

Statistical analysis. The data were analyzed with use of repeated-measure analysis of variance to test for overall differences between the observation times (baseline, 1, 6, and 7 months) for each parameter measured.

Table III. Tests of anticoagulation (mean ± SEM)

	Group	A: baseline value	B: 1-month therapy	C: 6-nonths the apy	D: 1-month after therapy	Stalistical significance at time intervals
Antithrombin III antigen	Smokers	26.4 ± 0.6	25.0 ± 0.5	25.1 ± 0.6	26.6 ± 0.7	B, C vs. D, A: $p = 0.03$
(normal: 22-39 mg/dl)	Over 34	27.3 ± 0.9	26.5 ± 0.9	26.6 ± 0.7	26.8 ± 0.8	B, C vs. D, A: $p = 0.03$
Antithrombin III activity	Smokers	104.3 ± 2.0	98.2 ± 2.5	97.3 ± 2.2	106.5 ± 1.4	B, C vs. D: $p = 0.005$
(normal: 30%-120% of normal)	Over 34	96.8 ± 3.6	98.6 ± 3.0	101.8 ± 2.2	100.2 ± 2.7	ŃS
α ₂ -Macroglobulin antigen	Smokers	262.4 ± 13.6	266.4 ± 14.4	272.4 ± 12.8	280 ± 13.0	NS
(normal: 175-450 mg/dl)	Over 34	247.1 ± 11.7	238.9 ± 11.7	236.9 ± 11.9	231 ± 10.2	NS
α ₂ -Antitrypsin antigen	Smokers	232.7 ± 10.6	263.2 ± 17.6	311.7 ± 13.6	246.7 ± 7.8	C vs. A, B, D: $p = 0.01$
(normál: 200-400 mg/dl)	Over 34	199.7 ± 13.3	250.0 ± 14.1	258.0 ± 11.7	196.3 ± 12	C vs. B, A, D: $p = 0.01$
Plasminogen antigen	Smokers	13.2 ± 0.5	20.0 ± 0.9	22.0 ± 0.9	14.8 ± 0.7	C vs. B, A, D: $p = 0.01$
(normal: 10-20 mg/ 100 ml)	Over 34	13.3 ± 0.6	16.3 ± 0.6	18.2 ± 0.5	13.8 ± 06	C vs. B, A, D: $p = 0.01$
Plasminogen activity (normal:	Smokers	3.2 ± 0.2	4.6 ± 0.2	4.8 ± 0.2	3.5 ± 0.2	B, C vs. A, D: $p = 0.301$
2.4-3.8 Committee on Thrombolytic Activity units/ml)	Over 34	3.4 ± 0.1	4.6 ± 0.3	4.6 ± 0.2	3.7 ± 0.2	B, C vs. A, D: $p = 0.301$
α ₂ -Antiplasmin activity	Smokers	97.8 ± 5.3	90.7 ± 6.3	100.4 ± 6.9	101.6 ± 6.3	NS
(normal: 80%-120% of normal)	Over 34	95.2 ± 6.5	82.6 ± 6.5	73.2 ± 4.8	100.9 ± 5.5	C vs. A, D: $p = 0.007$

If a significant time effect was noted, Tukey's Studentized range test, also known as the HSD (honestly significant difference) test, was used. If the HSD test failed to show any significant differences despite a time effect, Duncan's multiple range test was used to find which time points were significantly different. 9, 10

Results

Dynamic tests of coagulation and fibrinolysis were performed (Tables II and III). Those reflective of the coagulation cascade included the prothrombin time, thrombin time, and fibrinogen antigen.

Evidence of altered procoagulant activity was noted in the smokers: significant shortening of the prothrombin time but increases in the thrombin time and fibrinogen antigen occurred during treatment, with a return to baseline levels after the subjects had been off therapy for I month (Table II). A pattern similar to that of the smokers was noted in the older women, except that in the older group, both the partial thromboplastin time and the fibrinogen antigen levels remained unaffected. In each instance, the change in activity was still well within our normal range. Variability for each patient was similar to her group as a whole.

The results of the tests measuring fibrinolytic and inhibitory activity are summarized in Table III. Definite and important differences were noted between the smokers and the nonsmoking older group. When the smokers were considered as their own controls, a statistically significant decrease during treatment was recorded for antithrombin III, whether determined by antigen or activity methodology. Counterbalancing this effect, plasminogen antigen and activity were significantly increased to values in excess of the normal for our laboratory. The activity of the minor inhibitors was either unaffected (a2-macroglobulin) or moderately elevated (<2-antitrypsin).

The activity of the two major coagulation inhibitors in the women in group 2 was either not inhibited (antithrombia III activity) (Fig. 1) or significantly depressed (α₂-antiplasmin activity) (Fig. 2). An unexpected finding was the highly significant and progressive depression of a rtiplasmin activity from a baseline activity level of 95.2% \pm 6.5% to 82.6% \pm 6.5% and 73.2% \pm 4.8% after 1 and 6 months of treatment. The activity returned to 100.9% ± 5.5% 1 month after discontinuation of the oral contraceptive. Selected tests of fibrinolysis-plasminogen antigen and activity-showed increased evels, the latter at levels above the normal for our laboratory. As with the smokers, the minor protease inhibitors were either unchanged (α2-macroglobulin) or increased (a2-antitrypsin). With the exception of the measured decrease in antithrombin III antigen (values still within normal for our laboratory), all changes were indicative of an overall profibrinolytic effect. A causal relation:hip of these changes is demonstrated by the induced alteration during therapy with a subsequent return to basal levels I month after oral contraceptive adminis_ration.

Comment

To appreciate fully a true cause-and-effect relationship between oral contraceptive usage and thromboembolic disease, a few points relative to the coagulation cascade need to be considered. Many published studies report an increase in the concentration in a number of coagula ion factors associated with a "hypercoagulable" state. A. this time there are in fact no tests (single or

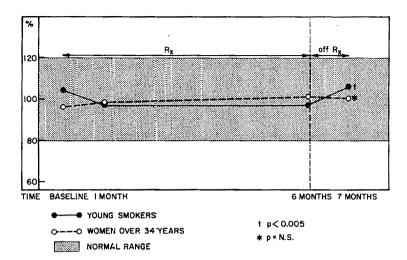


Fig. 1. The effect of age and smoking on antithrombin III activity in women using low-dose oral contraceptives.

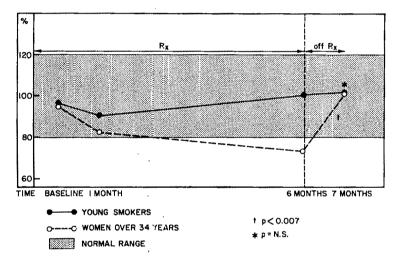


Fig. 2. The effect of age and smoking on antiplasmin activity in women using low-dose oral contraceptives.

in combination) that can determine hypercoagulability or can be used to predict who will experience a thromboembolic event. Further, there are no studies that have shown a cause-and-effect relationship between a procoagulant alteration in the coagulation profile and the development of a thrombus. Cognizance also needs to be taken of the overall effect: an elevation in factor X, for example, may be balanced by an increase in endogenous inhibitor (e.g., antithrombin III). It is fcr this reason that we have also monitored the so-called "dynamic" tests of coagulation-prothrombin time, partial thromboplastin time, thrombin time-rather than solely the assay of an individual clotting factor. In this context, statistically significant alterations in prothrombin time (0.0001), thrombin time (p < 0.03), and fibringen antigen (p < 0.0004) were noted in the smokers while using oral contraceptives. The prothrombin time was also shortened in the group 2 subjects, and the thrombin time increased. Similar changes were noted in healthy nonsmoking menstruating young women taking the same low-dose oral contraceptive. The direction of these results appears to be net procoagulant, but in each instance the change is slight and well within the range of normal for our laboratory. Although these changes are statistically significant, they are highly unlikely to be of any clinical importance. To date, there is no convincing evidence that an abnormally short prothrombin time, partial thromboplastin time, or thrombin time in and of itself predisposes one to thrombosis.

Scant attention has been paid to the effect of oral contraceptives on the fibrinolytic system. Plasminogen

is a \beta-globulin synthesized in the liver and as such is responsive to sex steroids.6 It was therefore not surprising to note the increase in plasminogen antigen and activity during contraceptive administration in both the smokers and the women over the age of 34. The clinical significance of elevated plasminogen is not known.

 α_2 -Antiplasmin (α_2 -plasmin inhibitor), on the other hand, is very potent, inhibiting 35% of plasmin generated from plasminogen.8 It has a well-recognized and important biologic role in hemostasis.8 An increase in plasmin activators—and therefore plasmin formation—or a decrease in a2-plasmin inhibitor, can lead to hemorrhage, but the critical level at which this occurs is not known." \alpha_9-Antiplasmin activity is increased during the luteal phase of the menstrual cycle, pregnancy,12 and, according to some authors, either unaffected12 or increased13 in women on combined oral contraceptives. The lowering of α₂-antiplasmin activity noted in our older subjects was an unexpected observation. Although the biologic meaning of the increase in plasminogen and decrease in α₂ antiplasmin in the over-34 age group is not clear, they would seem to suggest an overall increase in net fibrinolytic potential and hence perhaps security for women in this age group using oral contraceptives with this estrogen dosage.

Probably the most important endogenous coagulation inhibitor is antithrombin III. A number of studies have shown a statistically significant decrease in antithrombin III antigen and activity in women using combination oral contraceptives.4 However, the following four important points must be made:

- 1. Although reduced, the values have not been reduced to ranges consistent with spontaneous thrombosis (60% of normal).
- 2. The effect is dose related. Low-dose oral contraceptives containing 30 to 35 µg of ethinyl estradiol are associated either with no change⁶ or with a much-reduced effect when compared to the older higher-dose oral contraceptives.5,7
- 3. Antithrombin III should be assayed in plasma samples. Studies in which antithrombin III was measured in serum accounts for many of the reports showing a significant lowering of antithrombin III activity in oral contraceptive users.14
- 4. Most studies were performed in large groups of persons tested once rather than in a controlled manner as were our patients, who served as their own controls.

If one considers the statistically significant results in group 1, the smokers had evidence of procoagulant activity (prothrombin time, thrombin time, fibrinogen antigen), a decrease in their natural inhibitor activity (antithrombin III antigen and activity), no change in their fibrinolytic activity as measured by α2-antiplasmin activity, and an increase in plasminogen antigen and activity. The latter rose to values higher than normal for our aboratory, so it is possible that the oral contraceptive did induce a biologically significant effect. Of all risk factors associated with oral contraceptive use, smcking is consistently incriminated as having a marked adverse effect. According to a recent Royal College of General Practitioners report,15 death rates are increased synergistically in women who smoke and ingest oral contraceptives. The report cited an annual death rate in women below the age of 35 of one in 77,000 in nonsmokers and one in 10,000 smokers; the values for women aged 35 to 44 were one in 6750 and one in 2000, and for women over the age of 45, one in 2500 and one in 500, respectively.

Interpretation of statistically significant results should, nowever, be realistically evaluated in terms of their po sible clinical outcome. As illustrated in Fig. 1, although the level of antithrombin III in women smokers was significantly reduced (p < 0.005) after 6 months of contraceptive usage, the values were well within the range of normal and therefore unlikely to have any biologic effect. Conversely, α₂-antiplasmin activity in women over 34 years of age (Fig. 2) showed a statistical decrease to values below the normal for our laboratory; although not proved by this study, this change can be expected to have a biologic effect.

The overinterpretation of results based on laboratory tests has been compounded by contradictory reports from frequently quoted epidemiologic studies. For example, a report issued by the Royal College of General Practiticners in 1977 stated, "... in general it would be wise for all contraceptive users over 35 years of age to reconsicer their method of contraception . . . "15 Four years lazer a report from the same group concluded when referring to mortality rates in women between the age: of 35 and 45, "... these estimates permit a more flexible approach to women who are between 35 and 45 years of age. It is now apparent that the major risk occurs in smokers . . . "16

The secent introduction of the lower-dose oral contraceptizes and the observed decrease in venous thrombosis is compatible with the hemostatic profile noted in our older subjects. The unaltered antithrombin III activity, significantly increased plasminogen activity (above our laboratory's range of normal), and a marked decrease in \(\alpha_2\)-antiplasmin activity apparently provide an important safety factor against other possible procoagulant changes.

If the results of this study are confirmed, low-dose oral contraceptives could once again be considered for selected healthy, active nonsmoking women between the age: of 35 and 45. However, we believe that smoking, in and of itself, remains a relative contrainc cation to even the apparently safer, lower-dose oral zontraceptives.

The statistical analysis was performed by Jat 2 Pendergast, Ph.D., Department of Biostatistics, University of Florida College of Medicine. We wish to ackncyledge the technical assistance of Yvonne Suggs, R.N.

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Congenital herpes simplex type II infection

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A case of congenital herpes simplex virus type II infecton acquired in utero is reported. The issue of congenital infection due to the herpes simplex viruses ≡ discussed. (AM J OBSTET GYNECOL 1985;152: 1000-2.)

Key words: Herpes simplex, congenital infection, teratogen

Congenital infection due to the herpes simp-ex viruses is defined as the onset of the disease at the time of birth and within the first 48 hours following rupture of the fetal membranes. In contrast to neonataly ac-

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quired disease secondary to delivery and infected birth canal or ascending infection, congenital herpes is perceived to be a rare event. The rarity with which the herpes simplex viruses function as teratogens appears to be related to the likelihood that their presence relatively early in gestation usually results in abortion. The purpose of this report is to describe a case of congenital infection due to herpes simplex type II virus and to comment on the pathogenesis of fetal and neonatal disease due to this virus.

Case report

R. S., an unmarried 15-year-old, white, primigravid adolescent, presented at 19 weeks' gestation with multiple ulcerative lesions of the vulva. Viral cultures done

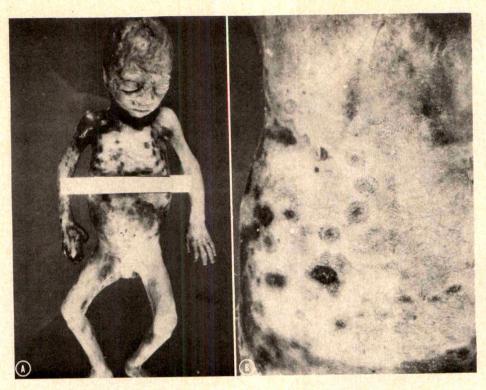


Fig. 1. A, Photograph of infant with vesicular and ulcerated lesions from which herpes simplex type II virus was isolated. B, Close photograph of vesicular and ulcerated lesions.

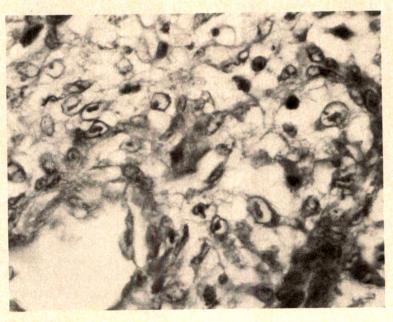


Fig. 2. Photomicrograph of lung exhibiting the presence of multiple intranuclear inclusion bodies. (Hematoxylin and eosin. Original magnification ×410.)

at another institution were positive for herpes simplex type II. The patient denied systemic symptoms and prior history of herpes or "cold sores." Her boyfriend had had a "cold sore" of the lip in recent months.

Although the lesions cleared in 1 week, cultures from the vulvovaginal area were positive at 10 days after onset of the disease but became negative 2 weeks later. She presented at 25 weeks' gestation complaining of leakage of fluid from the vagina. Rupture of the membranes was confirmed. Papanicolaou smear and cervical cultures failed to demonstrate the presence of virus. Twenty-four hours after the dissolution of the fetal membranes, the patient had spontaneous onset of labor and was delivered of a 520 gm female infant with obvious healing, fresh vesicular lesions of the skin (Fig. 1). The infant died shortly after birth.

Postmortem cultures from the skin, lung, and liver were positive for herpes simplex type II virus. Findings at autopsy included multifocal hepatic necrosis, acute and chronic inflammation of the pericardium, focal necrosis of the spleen, and meningoencephalitis. The intranuclear inclusion bodies characteristic of herpes simplex viruses were identified in cells marginal to the areas of coagulative necrosis (Fig. 2).

Comment

The well-developed histopathologic features, the absence of virus from the endocervix and posterior vaginal pool, and the fact that the fetal membranes had been ruptured only 24 hours largely precluded the possibility that the congenital herpes simplex type II infection was an ascending infection.

In utero infection can be the consequence of either hematogenous dissemination resulting from a maternal viremia or ascending infection associated with rupture of the fetal membranes. The reported case is an example of midtrimester hematogenous infection disseminated to the fetus and its biologic effect. To presume hematogenous dissemination, the infant has to have evidence of disease within 48 hours of rupture of the fetal membranes (the shortest known incubation time for the virus) or have a morphologic lesion older than that which could be accounted for by ascending infection.

There is limited acknowledgment in the English literature of congenital herpes infection as a valid disease entity. The magnitude of congenital disease may be underestimated for two reasons: (1) failure to attribute

teratogenicity and fetal wastage to the herpes simplex virus and (2) the probability that some cases of congenital disease manifest after the first 48 hours of life. In the absence of virologic studies, the cases cited by South et al.1 or by Schaffer2 would have been attributed to cytomegalovirus. South et al.1 reported a case of premature neonatal microcephaly, intracranial calcifications, microphthalmos, and retinal dysplasia associated with vesicular skin lesions from which herpes simplex virus type II was recovered. The mother and infant had antibody only to the type II virus. Schaffer2 reported a case of similar malformations that were also associated with herpesvirus isolated from skin lesions. These two cases associated with herpesvirus isolated from skin lesions suggest that herpes simplex virus type II early in gestation may exhibit a neurotrophic teratogenic effect. Gagnon³ reported a case of congenital herpes simplex infection that developed on day 4 of life. Had not the umbilical cord blood and placenta been cultured for virus at the time of parturition, a periparturitional acquisition of disease would have been inferred.

The case reported clearly indicates the ability of herpes simplex virus type II to infect the products of conception in utero and cause congenital infection.

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WARNINGS: Cigarette smoting increases the risk of serious cardiovascular side effects from OC use. This risk increases with age and with heavy smotlags (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use OCs should be strongly advised not to smoke.

not to smoke.
The use of OCs is associated with increased risk of several serious conditions including thromboembolism, stroke, myocardial infarction, liver tumor, gall bladder disease, visual disturbances, tetal abnormalities, and hypertension. Practitioners prescribing OCs should be familiar with the following information relating to these risks.

The use of Ocs is associated with increased risk of several serious conditions including thromboembolism, stroke, myocardial infarction, liver tumor, gall bladder disease, visual disturbances, fetal abnormalities, and hypertension. Practitiones prescribing Ocs should be familiar with the following information relating to these risks.

1. Thromboembolic Disorders and Other Vascular Problems: An increased risk of thromboembolic and thrombolic desease associated with OC use is established. One study demonstrated an increased relative risk for fatal venous thromboembolism. They serval usudes demonstrated of non-fatal venous thromboembolism. They serval usudes demonstrated on for non-fatal venous thromboembolism. They serval usudes demonstrated of non-fatal venous thromboembolism. They serval usudes demonstrated that they have been demonstrated of developing they have been demonstrated that they are control used to the control of the control

mortality is with the condom or diaphragm backed up by early abortion. The study-also concluded that OC users who smoke, especially over 30, have greater mediality lisk than OC users who do not smoke.

Table 1. Risk of thromboembolic and thrombolic disease associated with OCs increases with age after 30 and, for MI, is further increased by hypertension, hyperiprofemias, ebesity, diabetes, or history of preeclamptic toxemia, and especially as modifying the following chart gives a gross estimate of risk of death from circulatory disorders associated with OC use.

SMOKING HABITS AND OTHER PREDISPOSING

COMMUNITIONS—HISK ASSOCIATED WITH USE OF DCs						
Age	Below 30	30-39	40+			
Heavy smckers	С	В	A			
Light smokers	Ď	Č	Ĥ			
Nonsmokess		-	-			
(no predisposing conditions)	D	C D	c			
Nonsmoke:5	_					
(other pracisposing conditions)	C	C,B	B,A			

A—Use associated with very high risk B—Use associated with high risk. C—Use associated with moderate risk D—Use associated with low risk.

D—Use assiziated with low risk
Physician are pairent should be alert to earliest manifestations of thromboembolic and thrombook disorders (e.g., thrombophiebilis, pulmonary embolism, cerebrovasuiem smilliciency, coronary occlusion, retinal thromboss, and mesertenc thromboss). Should any of these occur or be suspected, disconnected the common of the c

out pregnancy before continuing the OC. If pregnancy is confirmed, tell the patient about potential risks to the fetus and discuss advisability of continuing the pregnancy whome who discussed the problem of the probl

Intraperitoneal immunotherapy of epithelial ovarian carcinoma with Corynebacterium parvum

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Los Angeles, California, and Boston, Massachusetts

Corynebacterium parvum was administered intraperitoneally to 21 patients with epithelial ovarian cancer. Nineteen patients had surgically measurable disease and two received adjuvant therapy. Surgically confirmed responses were documented in six of 19 patients (31.6%), with two complete responses (10.5%) and four partial responses (21.1%). Three patients (15.8%) had stable disease, and 10 patients (52.6%) had disease progression. The mean survival of the patients who had a complete response was 35.5 months; the four patients who had a partial response the mean survival was 26.6 months, and of the nonresponders the mean survival was 12.6 months (p < 0.02). The mean survival of the entire group was 18.2 months. Initial response and patient survival correlated with the amount of disease pretreatment. Thus six responding patients had ≤5 mm maximum diameter tumors, that is, minimal residual disease. Toxicity in the 86 courses of therapy included abdominal pain in 78% of cases, fever in 56%, nausea in 40%, and vomiting in 22%. Stimulation of cytotoxic lymphocytes resulted from the administration of C. parvum, which induced a significant increase of both intraperitoneal natural killer lymphocyte cytotoxicity and antibody-dependent cell-mediated cytotoxicity in six of nine patients tested; these two types of cytotoxicity correlated with response to therapy and may be partially responsible for the surgically documented tumor regression. While the clinical usefulness of intraperitoneal C. parvum is limited because of its toxicity, intraperitoneal immunotherapy may prove useful in patients with minimal residual ovarian cancer when more refined agents become available. (AM J OBSTET GYNECOL 1985;152:1003-10.)

Key words: Intraperitoneal immunotherapy, ovarian cancer, Corynebacterium parvum

It has been documented that intraperitoneal *Corynebacterium parvum* produces a significant antitumor effect in some patients who have minimal residual epithelial ovarian cancer found at second-look laparotomy. In addition, intraperitoneal immunotherapy with *C. parvum* has been effective in the palliation of ascites in patients with more advanced intra-abdominal disease. ²⁻⁴

From the Division of Gynecologic Oncology, Departments of Obstetrics and Gynecology, Microbiology and Immunology, University of California Los Angeles School of Medicine, and Jonsson Comprehensive Cancer Center; Division of Tumor Immunology and Medicine, Dana-Farber Cancer Institute, Boston, Massachusetts; and Department of Obstetrics and Gynecology, Division of Gynecologic Oncology, Brigham and Women's Hospital, Harvard Medical School.

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Reprint requests: Jonathan S. Berek, M.D., Division of Gynecologic Oncology, University of California (Los Angeles) School of Medicine, Los Angeles, California 90024. Patients with persistent epithelial ovarian malignancies documented at second-look laparotomy who have undergone combination cytotoxic chemotherapy are deficient in both natural killer and antibody-dependent cell-mediated cytotoxic effector lymphocytes in the peritoneal cavity.⁵ These lymphoid cells also show defective cytotoxic function in response to the in vitro agents that normally activate these cells, for example, *C. parvum* and interferons. This deficiency may be partially responsible for tumor proliferation and/or dissemination in the peritoneal cavity of these patients.

However, in patients who have minimal residual epithelial ovarian cancer documented at second-look laparotomy, the intraperitoneal instillation of multiple doses of *C. parvum* significantly activates both natural killer cytotoxic effector lymphocytes, and antibody-dependent cell-mediated cytotoxic effector lymphocytes in the peritoneal cavity. The activation of these peritoneal lymphoid cells may be responsible for tumor regression observed in some patients.

In our previous report of 11 patients with disease evaluable for response, five patients (45%) had surgically documented responses. The present report further documents the treatment outcome, toxicity, and

Table I. Characteristics of patients treated with intraperitoneal C. parvum

Patient	Histologic							
no.	Age	Stage	Туре	Grade	Largest residual tumor	Number of courses	Initial response	Follow-up
1	43	III	Serous	3	1-3 mm	4	CR	Died 10/82
2	45	III	Serous	3	5 cm	$\tilde{3}$	PD	Died 4/80
3	48	III	Serous	2	1-2 mm	4	CR	Died 8/82
4	41	IV	Serous	3	Positive cytology	8	SD	Died 11/81
5	44	Ш	Serous	3	NED	4	NED*	Alive with recurrence 2/84
6	47	IV	Poorly differ- entiated	3	4-6 mm nodules	3	PR	Died 9/82
7	40	Ш	Poorly differ- entiated	3	4-6 mm nodules	4	SD	Died 4/82
8	60	Ш	Undiffer- entiated	3	2 mm	3	PD	Died 9/81
9	50	III	Serous	3	NED	3	NED*	NED 10/84
10	46	111	Serous	3	2-3 mm	1	PD	Died 5/82
11	56	IV	Serous	3	1-3 mm	4	PD	Died 12/82
12	52	III	Poorly differ- entiated	3	Positive cytology	4	PD	Died 7/83
13	49	Ш	Mucinous	3	5-6 mm	4	SD	Died 9/84
14	41	III	Serous	2	Positive cytology	9	PD	NED 9/84
15	39	III	Serous	2	2-3 mm	7	PR	NED 9/84
16	34	Ш	Serous	3	4 mm	2	PR	Alive with recurrence 9/84
17	55	IV	Serous	3	3-4 mm	8	PR	Died 7/82
18	54	III	Serous	3	2-3 mm	8	PD	Died 7/84
19	51	III	Serous	2	>1.5 cm	4	PD	Died 1/84
20	.60	HC	Serous	3	>1.5 cm	3	PD	Died 6/83
21	47	III	Serous	3	>1.5 cm	2	PD	Died 9/83

CR = Complete response; PR = partial response; SD = stable disease; PD = progression of disease; NED = no evidence of disease.

survival of the 21 patients who have now received intraperitoneal *C. parvum* in this phase I-II "salvage" treatment regimen for advanced epithelial ovarian cancer.

Material and methods

Patients. A total of 21 patients were treated with intraperitoneal *C. parvum*, with use of protocols approved by the Human Subject Protection Committees of the University of California (Los Angeles) School of Medicine, the Dana-Farber Cancer Institute, and the Brigham and Women's Hospital. All 21 patients were evaluable for studies of toxicity, and 19 patients were surgically evaluable for response to *C. parvum*. Characteristics of each patient are listed in Table I.

All patients underwent a second-look laparotomy or laparoscopy at the University of California (Los Angeles) or the Brigham and Women's Hospital before treatment with intraperitoneal *C. parvum*. Treatment catheters were placed at the time of surgical exploration as described below. No patient had evidence of disease outside the peritoneal cavity documented by physical examination, chest x-ray, and computerized axial tomography of the pelvis and abdomen.

Patients ranged in age from 35 to 60 years with a median age of 48 years. Before initial therapy one patient was classified as International Federation of Gynecology and Obstetrics Stage IIC, 16 patients as Stage III, and four patients as Stage IV, based on small pleural effusions that disappeared in response to chemotherapy. At the initiation of intraperitoneal therapy, no patients had documented extraperitoneal disease.

Residual tumors before *C. parvum* therapy ranged from microscopic to 5 cm in maximum diameter. Three patients (15.8%) had microscopic disease defined by positive peritoneal cytologic findings, 10 patients (52.6%) had measurable disease ≤5 mm maximum diameter, three patients (15.8%) had disease ranging from >5 mm to 1.5 cm, and three patients (15.8%) had disease >1.5 cm. Additionally, *C. parvum* was used as adjuvant therapy for two patients who had no evidence of disease at second-look laparotomy. Papillary serous cystadenocarcinoma was the predominate histologic type. Most tumors were poorly differentiated; 17 of 21 were grade 3 (of 3 grades).

Before immunotherapy each patient had undergone 6 to 12 months of intravenous treatment of combination chemotherapy with cyclophosphamide, doxorubi-

^{*}Adjuvant treatment.

cin, and cisplatin, followed by a second-look laparotomy or laparoscopy, at which time 1 or 2 Tenckhoff catheters were placed surgically. The reassessment operations were performed 4 to 6 weeks following the final course of *C. parvum* to evaluate response in those patients who remained clinically free of disease at the completion of therapy.

Intraperitoneal catheters. Tenckhoff peritoneal dialysis catheters were used for administration of intraperitoneal immunotherapy. The indwelling catheter facilitated repeated administration of *C. parvum*, multiple sampling of peritoneal washings for cytology, and cytotoxicity assays of peritoneal lymphoid cells.

A standard operative technique was used for insertion of the catheters. With the patient under general anesthesia, a transverse incision was made in the midline of the abdomen. The technical details for the insertion of the dialysis catheter have been previously described. The Tenckhoff catheter is inserted with the tip of the catheter directed toward the pelvis. When a second catheter was placed, its tip was directed cephalad in the right paracolic gutter. The free end of the Tenckhoff catheter was tunneled through the superficial fascia and brought out through a small stab incision about 10 cm lateral to the midline.

Dialysis fluid was instilled into the abdominal cavity (Impersol with 1.5% dextrose, Abbott Laboratories, North Chicago, Illinois, or Dyalasate with 1.5% dextrose). The fluid had been warmed to 37° C, supplemented with 500 U/L of sodium heparin (Panheparin, Abbott Laboratories) and 4 mEq/L of potassium chloride.

After the operation, intermittent peritoneal lavage was performed with use of 1 to 2 L of warmed dialysis fluid until the peritoneal washings were free of blood. Lavage was performed every 4 to 8 hours until the fluid was clear, which usually took 2 to 3 days. Thereafter, lavage was performed daily until the first *C. parvum* treatment, 7 to 10 days after insertion of the Tenckhoff catheter(s).

Following treatment with *C. parvum*, the catheter was flushed daily with 50 ml of saline solution and 10 ml of heparinized saline solution. Lavage was performed twice weekly with 1 L of warmed dialysis fluid to maintain patency of the peritoneal catheters. At each lavage, fluid was examined cytologically for malignant cells, and cultures were taken. Distribution of dialysis fluid throughout the peritoneum was confirmed by radionuclide scintigraphy with use of technetium 99m sulfur colloid.

C. parvum administration. C. parvum (Coparvax, Burroughs Wellcome, Research Triangle Park, North Carolina) was administered intraperitoneally every 2

weeks. The dosage of *C. parvum* was initially 250 μ g/m², which was repeated for the second cycle, and then escalated by doubling the dose to 500 μ g/m², 1000 μ g/m², 2000 μ g/m², and 4000 μ g/m² until a maximally tolerated dose was attained, and/or four symptomatic courses had been given. During the study the maximum dose administered was 4000 μ g/m², and the maximum number of courses was nine.

Thirty minutes before each administration of *C. parvum*, patients were premedicated with 50 mg of diphenhydramine hydrochloride and 650 mg of acetaminophen orally. The latter was given every 4 hours for the first 48 hours after treatment. Following premedication, 2 L of dialysis fluid was instilled into the abdominal cavity and recovered for cytologic and immunologic studies. Then 1750 ml of warmed dialysis fluid was instilled, and *C. parvum* in 250 ml normal saline solution (Abbott Laboratories) was infused into the peritoneal cavity over 1 hour. The exteriorized ends of the Tenckhoff catheters were aseptically resealed. Toxicity was monitored as previously described.

Evaluation of response to therapy. Cells for cytologic analysis and immunologic assays were obtained at least weekly by peritoneal lavage. Blood samples were obtained weekly for immunologic assays.

A reassessment laparoscopy or laparotomy ("third-look") was performed at the conclusion of intraperitoneal *C. parvum* treatment in all patients who were clinically free of disease. In order to accurately document surgical response, all patients had their operations carried out at either the University of California (Los Angeles) or at the Brigham and Women's Hospital by the same surgeons who performed the initial operation. The technique of second-look laparotomy had been described by members of our groups.^{7,8} In patients without macroscopic evidence of disease, peritoneal washings were examined for cytologic evidence of malignancy and multiple intraperitoneal biopsies were performed.

The duration of response was measured from the initiation of immunotherapy. Survival is measured from both the initial diagnosis and from the initiation of immunotherapy to the last patient follow-up or death. Standard actuarial life-table analyses were used for survival calculations⁹ and tests of significance.¹⁰

Separation of peritoneal cells. Peritoneal lavage was performed twice weekly after *C. parvum* administration with use of 2 L of dialysis fluid, which was instilled intraperitoneally as previously described. Then approximately 500 to 1000 ml were withdrawn for cytologic assays and determinations of natural killer and antibody-dependent cell-mediated cytotoxicity. Cells were centrifuged at 300 g for 10 minutes, washed, re-

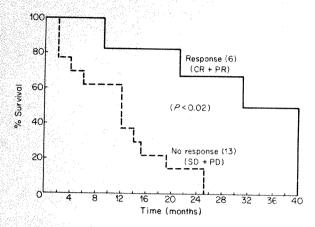


Fig. 1. Survival of patients treated with intraperitoneal C, parvum according to response is presented. Six patients who responded (CR + PR), complete plus partial response; old line) had a mean survival of 30.2 ± 5.5 months compared to 13 nonresponding patients (SD + PD), stable disease plus progression of disease; broken line) whose mean survival was 12.6 ± 2.9 months (p < 0.02).

suspended in RPMI medium, and examined with Wright's stain for differential counts. Lymphocytes were isolated by Ficoll-Hypaque density gradient, incubated with carbonyl iron, and separated over a magnet.⁵

Natural killer lymphocyte cytotoxicity. Natural killer cytotoxicity was evaluated by a previously described procedure. ^{5, 6, 51}Cr—labeled K562 target cells (1 × 10¹) were mixed with varying numbers of separated peritoneal lymphocytes, at effector-to-target ratios of 25:1 and 10:1. Cultures were incubated in V-well microtiter plates for 3 hours at 37° C. Supernatant was collected and ⁵¹Cr release assayed by gamma emission. Percent of lysis was calculated as follows (cpm = counts per minute):

Addition of detergent (Triton X) evaluated maximal release, while culturing of target cells alone served as background release.

Antibody-dependent cell-mediated cytotoxicity. The technique for evaluation of blood and pentoneal antibody-dependent cell-mediated cytotoxicity has been previously reported. An epithelial ovarian carcinoma cell line (OVCA433) was used as the tumer target in all assays. Targets were labeled with Tr by incubation at 37° C for 30 minutes in culture medium. After labeling, tumor cells were washed twice in medium without Tr and incubated for 30 minutes at 37° C with nonimmune rabbit serum or absorbed rabbit

Table II. Survival of patients receiving intraperitoneal *C. parvum* according to response status

Initial response	Patients (n)	%	Surviving (n)	Survival (mo)*
Complete	2	10.5	0	35.5 ± 4.5
Partial	4	21.1	2	26.5 ± 8.7
Stable	3	15.8	$\bar{0}$	19.7 ± 3.4
Progression	10	52.6	ĭ	9.8 ± 2.9
Total	19	100.0	3	18.2 ± 3.2

^{*}Measured from initiation of immunotherapy with C. parvum to last follow-up or death of patient; mean \pm SE.

heteroantiserum generated as previously outlined. Target cell suspensions were added to microtiter plates containing peritoneal cells at effector-to-target ratios of 25:1 and 10:1 and centrifuged at 250 g for 7 minutes, 100 µl samples of supernatant were removed for assay in a gamma counter. The activity of ⁵¹Cr released into the supernatant during incubation was expressed with use of the same formula outlined above for natural killer cytotoxicity. Spontaneous background release was determined by target cells in the presence of antibody but without effector cells. Maximum release was evaluated by the addition of detergent.

Results

Response to immunotherapy. Two patients had complete surgical responses documented by laparotomy (Table II). Both patients initially had small macroscopic disease measuring 1 to 3 mm in maximum diameter. Mean survival time (from initiation of immunotherapy to death from disease) for patients with a complete surgical response was 35.5 ± 4.5 months. Both of these patients had received four courses on intraperitoneal *C. parvum*.

Partial surgical responses were observed in four patients. The mean survival for this group was 26.5 ± 8.6 months. Of the two surviving patients who had a partial response, one is clinically free of disease 38 months following therapy and the other is alive but developed a recurrence in the subcutaneous tissue of the anterior abdominal wall 24 months following therapy, though there is no other evidence of systemic disease.

Overall, the mean survival from time of treatment of patients who responded was 30.2 ± 5.5 months compared to a mean survival of 12.6 ± 2.9 months for patients who did not respond (p < 0.02) (Fig. 1). The mean survival of the entire patient group was 18.2 months.

All six patients who responded to treatment had minimal residual disease of ≤ 5 mm maximum tumor diameter (Table III). No response was seen in the five

Table III. Survival and response	status of patients	receiving intrape	ritoneal C. parvum	by maximum
residual disease diameter				

residual disease diasie		Complete response plus partial response		Stable disease		Progression of disease		Survival	
Residual disease	Evaluable patients (n)	n	%	n	%	n	%	(mo)	
Microscopic >Micro. to 5 mm >5 mm to 1.5 cm >1.5 cm	3 11 2 3	0 6 0 0	0 55 0 0	1 0 2 0	33 0 100 0	2 5 0 3	67 45 0 100	31.4 ± 8.3 21.0 ± 4.5 18.0 ± 3.0 2.7 ± 0.7	
Total	19	6	31.6	3	15.8	10	52.6	18.2 ± 3.2	

patients whose tumors were >5 mm maximum tumor dimension. Longer survivals were also documented in patients with smaller tumor dimensions (Fig. 2). The mean survival status of patients according to initial tumor burden is also listed in Table III.

Three patients had stable disease during treatment, and their mean survival was 19.7 ± 3.4 months. Ten patients had disease progression, and their mean survival was 9.8 ± 2.9 months.

The two patients in whom *C. parvum* was used as adjuvant therapy are alive: one patient is free of disease 41 months following treatment, and one has had a recurrence clinically after 42 months.

Toxicity. Side effects were monitored in 21 patients, who received a total of 86 courses of intraperitoneal immunotherapy (Table IV). Abdominal pain occurred in 78% of courses and often required narcotic analgesics for relief. Fever (>38° C) was associated with 56% of treatment courses, despite the prophylactic administration of oral acetominophen. Nausea occurred in 40% and vomiting in 22% of courses, but these were controlled with antiemetics and were dose limiting in only one patient. Hypotension was associated with seven courses (8%) and appeared to be related to rapid fluid shifts into the peritoneal cavity. In five of these instances the patients had been receiving propranolol or levantradol, which could have compromised the patient's ability to compensate for the acute hypovolemia. However, in each case the hypotension responded promptly to the intravenous administration of crystalloids, and vasopressor medications were not required.

Several complications were related to use of the Tenckhoff catheters. Two episodes of infection were noted. In one patient the catheter was colonized with *Staphylococcus epidermidis* without signs of intraperitoneal infection; in a second patient, overt peritonitis developed secondary to a *S. aureus* infection. Both patients responded promptly to antibiotic therapy.

There have been two episodes of significant intraperitoneal bleeding. In one patient an asymptomatic hematoma was discovered at the time of a second-look laparotomy. A hematoma had formed at the site of the Tenckhoff catheter, which had eroded into the mesentery of the ascending colon. To avoid potential compromise of the colonic vasculature and to rule out persistent malignancy, a segmental resection of the affected portion of the large bowel was performed. The patient recovered without subsequent complications. In a second patient an episode of intra-abdominal bleeding resulted from diffuse peritonitis not associated with infection. At exploratory laparotomy the bleeding was easily controlled by electrocautery.

The extent of adhesion formation varied considerably. In one patient, repeated intraperitoneal administration of *C. parvum* was associated with severe adhesions. In two patients, adhesions formed after the insertion of the Tenckhoff catheter and before intraperitoneal instillation of *C. parvum*. In most other patients who had not formed extensive adhesions following previous surgery for their ovarian tumors, only minimal adhesions developed in response to the subsequent intraperitoneal *C. parvum*. However, loculation of dialysis fluid or clogging of the catheter required replacement of the Tenckhoff catheter in six of the 21 patients (28.6%).

C. parvum administration was not associated with hematologic, renal, hepatic, or neural toxicity. No acute or chronic systemic hypersensitivity reactions were encountered.

Host cells infiltration of the peritoneal cavity. After the intraperitoneal injection of *C. parvum*, there was a marked increase in the number of cells that could be lavaged from the peritoneal cavity. ^{1, 6} In the initial 24 to 48 hours following treatment, polymorphonuclear leukocytes predominated, but over the next 7 to 14 days mononuclear phagocytes represented the predominant cell type in the peritoneal cavity.

Natural killer cytotoxicity. Before treatment, all patients who were evaluated demonstrated low levels of peritoneal natural killer cytotoxicity. Following intraperitoneal *C. parvum* treatment, six of nine patients tested (67%) exhibited an increase in natural killer lym-

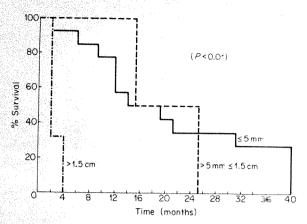


Fig. 2. Survival of patients compared to the maximum amount of residual tumor at the initiation of intraperitoneal immunotherapy is presented. The mean survival of patients whose maximum disease was ≤ 5 mm in largest diameter was 18.0 ± 1.9 months (——); versus 18.0 ± 3.0 months for patients whose tumors were between >5 mm ≤ 1.5 cm (——); and 2.7 ± 0.7 months for patients with tumors >1.5 cm \leftarrow —). There was no statistical difference between the survival of patients in the group with minimal residual disease compared to those with optimal tumor status (≤ 1.5 cm disease). However, both of these survival curves were significantly different from those of patients with nonoptimal disease (>1.5 cm maximum tumor diameter, p < 0.01).

phocyte cytotoxicity. Two patients showed a boost of natural killer cytotoxicity following treatment with a dose level of 250 µg/m² of *C. parvum* as well as two patients after 500 µg/m² and two patients after 1000 µg/m². The maximal level of increase in natural killer cytotoxicity was noted on day 7 following treatment; by day 14 the peritoneal natural killer activity had decreased to baseline levels in all patients.

Of the six patients who exhibited significant boosts in natural killer cytotoxicity, three patients responded to intraperitoneal therapy (one complete response. two partial), one patient had stable disease, one remained free of disease following adjuvant therapy, and one developed disease progression. Of the three patients whose natural killer cytotoxicity remained at low levels in the peritoneal cavity, none responded to intraperitoneal therapy; one had stable disease, and two had disease progression.

Although most patients demonstrated a modest increase in blood natural killer levels, there were no consistent changes in these values.

Antibody-dependent cell-mediated cytotoxicity. Nine patients had evaluation of peritoneal antibody-dependent cell-mediated cytotoxicity during therapy. The highest pretreatment values for each patient ranged from 0% to 7% specific lysis at 4 hours. A significant increase in antibody-dependent cell-mediated cytotoxic activity was observed in six of nine patients

Table IV. Toxicity from intraperitoneal *C. parvum* (86 courses)

	Occurrence of side effects				
	n	76			
Abdominal pain	67	78			
Fever (>38° C)	48	56			
Nausea	34	40			
Vomiting	19	22			
Hypotension	7	8			
Infection	2	2			
Bleeding	2	2			

(67%) during the first four courses of treatment. The most impressive and consistent increase in activity was observed in one of the complete responders. No patients with stable or progressive disease had a significant boost of intraperitoneal antibody-dependent cell-mediated cytotoxic activity.

Blood antibody-dependent cell-mediated cytotoxic measurements did not generally correlate with the time of treatment or with the response, although some patients exhibited modest boosts of the cytotoxicity.

Comment

Since the persistence of cancer documented at second-look laparotomy for patients with epithelial ovarian malignancy is 60% to 70%,⁷ the need for improved primary or secondary therapies is crucial if survival and cure rates are to be improved for this disease.

Second-line or "salvage" chemotherapies have generally been unsuccessful when administered systemically. Therefore, newer therapeutic approaches are being investigated. Intraperitoneal therapy is theoretically appealing, because the therapeutic agent can be brought into direct contact with the tumor cells in the peritoneal cavity, and much higher concentrations of drugs can be delivered. Biologic response modifiers have been most successfully employed when the agent is brought into direct contact with the tumor cells, either by intralesional or intracavitary injection and when the initial tumor burden is minimal. 11

The use of intraperitoneally administered *C. parvum* as a biologic response modifier is based on extensive preclinical data generated in our laboratories with use of a C3HeB/FeJ murine ovarian cancer model. This ovarian cancer model permits the evaluation of the intraperitoneal instillation of biologic response modifiers and other drugs. ¹²⁻¹⁴ Tumor regression and prolonged survival occurred when *C. parvum* was administered intraperitoneally to mice bearing ovarian tumor transplants. The fraction of long-term survivors correlated with the dose administered. Moreover, intraperitoneal

C. parvum instillation was effective against 105 tumor cells but not against 106.

Our present report of this phase I-II clinical study of the intraperitoneal instillation of repeated and escalating doses of C. parvum documents tumor regression in patients with minimal residual ovarian cancer (≤5 mm maximum diameter) in the peritoneal cavity. Patients whose tumors were of larger dimension did not respond to this immunotherapeutic approach, even when the tumor was in a single location.

Patients who responded to intraperitoneal C. parvum had a longer survival from the time of treatment compared to patients who did not respond. However, since overall survival from the initial diagnosis to final followup did not differ statistically for the two groups, the treatment per se may not have influenced survival.

In patients who responded to intraperitoneal C. parvum, peritoneal lymphocytes exhibited increased natural killer and antibody-dependent cell-mediated cytotoxicity. Although a positive trend existed, the numbers of patients in this series are too few to establish any significant correlation. These effector cells may be partially responsible for tumor regression in these patients. 1.3.6 Simultaneous measurements of blood natural killer and antibody-dependent cell-mediated cytotoxicity demonstrated that blood values did not correlate with response and that the immunologic induction of cytotoxic lymphocytes occurred principally in the peritoneal cavity. This may be significant, because the activation of regional intraperitoneal effector cells may be necessary for tumor control and may in part explain the mechanism of action of intracavity biologic response modifier administration. These data may also help to explain why most trials of systemically administered biologic response modifiers have produced only an occasional complete clinical response.11

The toxicity of intraperitoneal C. parvum was significant and the technique is somewhat cumbersome, because the catheters require surgical placement and daily maintenance. The instillation of this agent induces an intense inflammatory response, which is probably responsible for its antitumor activity, but it also tends to produce intraperitoneal adhesions, resulting in morbidity and catheter dysfunction. These features limit the potential clinical usefulness of this immunotherapeutic approach.

Since the intraperitoneal administration of a biologic response modifier can produce tumor regression in some patients with minimal tumor burden, more refined and less toxic agents need to be developed for this purpose. Biochemically treated fractions of nonspecific biologic response modifiers are being developed and, in a murine system, appear to have augmented antitumor activity with lower morbidity compared to C. parvum.14 Also, these immunologic agents may prove to be synergistic with cytotoxic agents.15

Because of recombinant genetic technology, large amounts of biochemically pure biologic response modifiers are becoming available. In particular, recombinant interferons, which are potent activators of cytotoxic lymphoid effector cells, are now available for clinical trials. A recent phase I-II trial of intraperitoneal α-recombinant interferon (rINF-α2) suggests that this agent can also produce tumor regression in patients with small residual tumors and that the toxicity of this agent is less than that with C. parvum.16

In the future intraperitoneal immunotherapy may prove useful as adjuvant treatment in patients with epithelial ovarian cancer in histopathologic remission or effective in combination with cytotoxic chemotherapy, which is clearly immunosuppressive in the peritoneal cavity of patients with ovarian cancer.5

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Surgical problems in the management of giant fibroadenoma of the breast

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Giant intracanalicular fibroadenoma is reported to be the most common cause of a massive, deforming enlargement of one breast in the female adolescent. It occurs infrequently and has a varied histopathologic pattern. It is suggested that obstetricians and gynecologists should have an awareness of this potentially malignant tumor of the breast with its associated problems of diagnosis and surgical management. Consideration of these huge breast tumors in relation to other similar breast lesions is presented in order to emphasize differential characteristics and treatment. (AM J OBSTET GYNECOL 1985;152:1010-5.)

Key words: Giant fibroadenoma, female adolescents, cystosarcoma, fibrosarcoma

Giant fibroadenoma of the breast is a fibroadenoma of large size that involves the major portion of the breast. The clinical features, the pathologic patterns, and the surgical management of representative cases of giant intracanalicular fibroadenoma are presented.

Case reports

Case 1. In 1943 O. W., a black female. 17 years of age, presented with a massive, painful enlargement of the right breast which had developed over a 2-year period. No discharge was noted. The patient's general health was good. Menstruation was normal.

The physical examination was negative except for the massive enlargement of the right breast (Fig. 1). Dilated superficial veins were noted in the skin. The nipple was distorted. The large, firm tumor was considered to be a giant fibroadenoma. The left breast and the axillary lymph nodes were negative to palpation.

At the operation an enormous, apparently benign, encapsulated tumor, which seemed to involve the entire breast, was removed through a semilunar, inframammary incision. The postoperative course was unevent-

ful. The gross examination of the breast specimen revealed a large, gray-tan, encapsulated tumor that involved the entire breast. Histologic studies of the tumor disclosed an intracanalicular and pericanalicular fibroadenoma without evidence of malignant neoplasia.

The patient was lost to follow-up in the dispensary clinic.

Case 2. In 1975 E. W., a 25-year-old black woman had a giant-size intracanalicular fibroadenoma (Fig. 2) removed at another hospital through a transverse, elliptical incision that sacrificed the nipple-areolar complex. The postoperative course was uneventful. Pathologically, the tumor was benign, weighed 4½ pounds, and was 16 cm in diameter. It was lobulated and rubbery and was composed of a white, fibrous, trabecular component and a yellow, slightly bulging, lobulated component. The histologic appearance of the tumor was similar to that of an intracanalicular fibroadenoma without evidence of malignancy (Fig. 3).

Five years after surgical removal of the breast lesion, the patient requested reconstruction. At this time, there was no evidence of recurrent tumor. Breast reconstruction with a silicone gel implant was deferred for various reasons.

Case 3. In 1978 K. R., a 23-year-old black woman, was initially treated at another medical institution for a massive enlargement of the right breast. Her general health was good. Menstruation was normal.

In an effort to reduce the size of the breast, a reduction mammoplasty was recommended and performed by members of the plastic surgical section.

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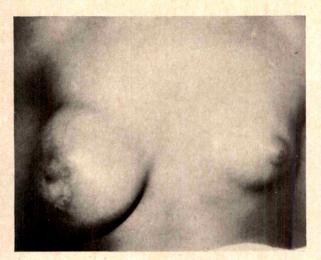


Fig. 1. Enlargement of the right breast in a 17-year-old girl caused by a giant intracanalicular fibroadenoma.

Transplantation of the nipple-areolar complex resulted in necrosis and subsequent development of a white scar in the center of the breast. A fibroadenoma was also removed from this breast.

Two years following the reduction mammoplasty, the patient, now 25 years of age, complained of a persistent enlargement of the right breast as well as some lumps in the enlarged left breast. The right breast was enlarged by a huge, firm mass that occupied the greater part of the breast. A white, circular scar was prominently observed in the middle of the breast. Numerous, movable, walnut-size fibroadenomas were present in the enlarged left breast (Fig. 4).

Surgical treatment was recommended. Through a curved submammary incision, an enormous, encapsulated, multilobular tumor was delivered and removed with some difficulty because of the previous plastic operation. Two fibroadenomas were removed from the left breast. The postoperative course was uneventful, with discharge from the hospital on the fifth day after

The surgical specimen from the right breast consisted of a large, multilobulated tumor, weighing 1423 gm and measuring $21 \times 16 \times 11$ cm (Fig. 5). The appearance of this tumor was consistent with a fibroadenoma. The encapsulated tumors removed from the left breast resembled fibroadenomas. The histologic examinations of the tumors from each breast showed a mixture of intracanalicular and pericanalicular structures with a bland, collagenous stroma. No malignant change of the epithelium was noted in multiple sections.

Two years later the patient returned for further surgical treatment of newly formed fibroadenomas in the right breast. Numerous, movable tumors, which were encountered in the enlarged left breast, were characteristic of solitary fibroadenomas.

Surgical intervention was again recommended. At the operation, newly developed fibroadenomas were removed from minimal residual tissue in the right breast. Since the left breast was enlarged as a result of



Fig. 2. Massive enlargement of the right breast caused by a giant-size fibroadenoma in an 18-year-old woman. The tumor weighed 41/2 pounds.

multiple fibroadenomas, and because of the possibility of further recurrence of additional tumors, whether benign or malignant, the patient consented to the removal of all breast tissue. A left subcutaneous mastectomy was performed. Reconstruction of both breasts was accomplished with silicone gel implants (250 cc) (Fig. 6). Meticulous care was given to each breast wound.

The gross examinations of the surgical specimens from each breast were compatible with benign fibroadenomas. Many histologic sections were described as being consistent with pericanalicular and intracanalicular fibroadenoma without evidence of pleomorphism (Fig. 7, A and B).

The patient was lost to follow-up after discharge from the hospital.

Comment

Individual case reports have appeared in the surgical literature illustrating the problems associated with massive breast tumors in young women, but the report by Owens and Adams1 on the subject of giant fibroadenoma tends to clarify the confusion regarding the character of this mammary tumor, both from the multiplicity of names it has received and from the variations in histologic structure. These authors urged the acceptance of the simple descriptive name "giant intracanalicular fibroadenoma of the breast."

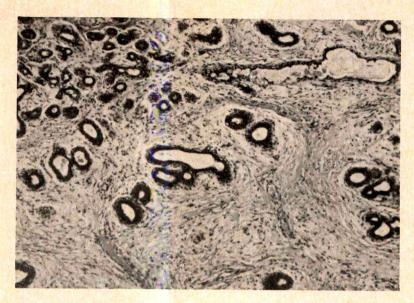


Fig. 3. Microscopic sections of the breast tumor shown in Fig. 2 reveal many ducts surfaced by hyperplastic, cuboidal epithelium embedded in a cellular fibrous tissue stroma. No nuclear pleomorphism was observed.

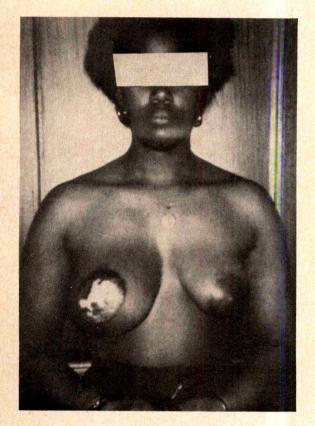


Fig. 4. Massive enlargement of the right breast caused by a huge fibroadenoma in a 23-year-old woman. The left breast is enlarged as a result of the presence of multiple fibroadenomas.

A fibroadenoma is the third most common tumor of the breast in present-day American women, being exceeded in frequency only by cystic disease or by carcinoma, according to Haagensen. These tumors are frequently multiple, developing concurrently or successively in one or both breasts. The massive fibroadenoma of youth may originate from a solitary lesion in one breast before or after the onset of menstruation. As they reach a substantial size and are removed, new ones may develop, as mentioned in Case 3. As long as any mammary tissue exists, the process may continue. These lesions are often compared to a cystosarcoma because they grow rapidly and attain a large size.

The giant-size fibroadenomas are gray-white, encapsulated, and firm; they may form a whorl-like pattern and may deform the breast as well as occupy the greater part of it.² The microscopic pattern is usually comparable to that of an ordinary fibroadenoma; however, it may vary because of different amounts of the two components of the tumor, namely, a proliferating connective tissue stroma and an atypical multiplication of ducts and acini. Occasional cases have been reported in which the epithelial element of a fibroadenoma has become neoplastic, but these instances are extremely rare.³

The term "giant intracanalicular fibroadenoma" may often be confused with the term "benign cystosarcoma phyllodes," so named in 1838 by Johannes Müller, who described this tumor as cystic and phylloid, or leaflike, because of the branching projections of tumor tissue into the cystic cavities within the tumor.

Benign cystosarcoma phyllodes is closely related to the fibroadenoma and is distinguishable from it in that the fibrous stroma is more cellular. Haagensen⁵ divided fibroepithelial tumors into two groups based on their histologic characteristics: adenofibroma and cystosarcoma phyllodes. He indicated that if the stroma is very cellular and made up of cells that vary considerably in size and shape and have hyperchromatic nuclei with a

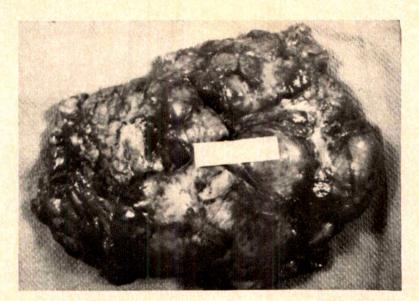


Fig. 5. Note the large, multilobulated fibroadenoma removed from the right breast in Fig. 4. This extraordinary, gray-tan fibroadenoma weighed 1423 gm.

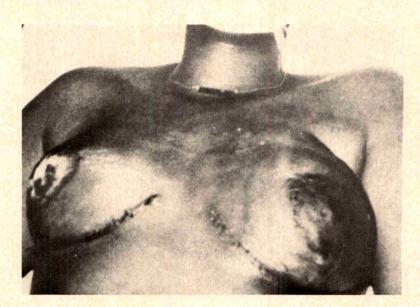


Fig. 6. Postoperative view of the patient in Fig. 4 showing the appearance of the breasts following subcutaneous mastectomy and prosthetic reconstruction.

significant number of mitoses—a stroma that suggests a malignant character—the lesion is considered to be a cystosarcoma.

Cystosarcoma may arise from a preexisting adenofibroma, according to Haagensen.5 These tumors are large and produce a characteristic teardrop appearance to the breast. They are well delineated but have no true capsule. They vary in color and are softer than a fibroadenoma. The overlying skin is unattached. Microscopically these tumors show extensive stromal cellularity and intracanalicular invasion, in addition to occasional myxoid, degenerative, and cystic changes. The most impressive kind of evidence that cystosarcoma and fibroadenoma are in some way associated is the frequent microscopic findings of both lesions in the same tumor.

The glandular epithelial elements of the cystosarcoma rarely undergo malignant degeneration. Local recurrences are likely to appear because of inadequate excision; however, malignant cystosarcomas, when they occur, may metastasize, after all efforts to control the disease locally, through the blood stream and only with rarity to the regional lymph nodes. Haagensen⁵ emphasized that if it were possible to predict which ones will metastasize, it might be justifiable to treat selected patients by mastectomy. Wide local excision may be successful in removal of most instances of benign cystosarcoma.

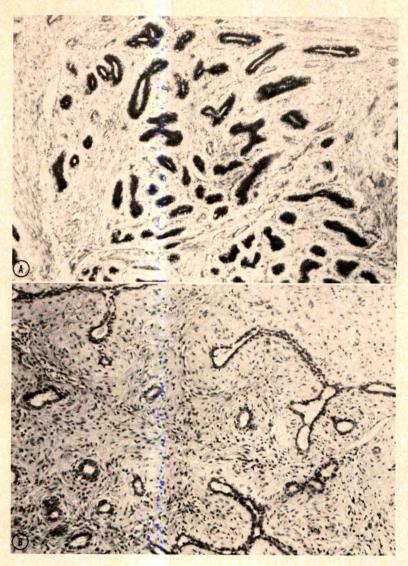


Fig. 7. A, Microscopic examination of the right breast tumor in Fig. 5 revealed a mixture of intracanalicular and pericanalicular fibroadenoma in an abundant collagenous stroma. No cellular pleomorphism was observed. B, Note the histologic appearance of one of the solitary fibroadenomas removed from the left breast.

A wide variety of types of sarcoma-like metaplasia are noted in the stroma of cystosarcoma. The most frequent type suggests a fibrosarcoma. True fibrosarcomas of the breast may occur, but they are rarely encountered. They may form a large mass in the breast. Microscopically they are composed of a stroma occupied by widely disseminated hyperchromatic spindle-shaped cells with abundant mitoses. Metastases from these tumors are usually blood borne. Other types of sarcoma, such as liposarcoma, hemangiosarcoma, and lymphosarcoma, rarely occur within the breast.

McDonald and Harrington⁶ stated that cystosarcoma phyllodes implies a large tumor of the breast which is a benign fibroadenoma in approximately 90% of instances and a fibrosarcoma in the remainder. Treves and Sunderland,⁷ in a review of a large series of cases

of cystosarcoma phyllodes, treated in Cancer Memorial Hospital in New York, emphasized that the simple criteria used to differentiate these tumors from fibroadenoma is the pronounced overgrowth of the stromal component. They recommended radical mastectomy for all of these tumors containing histologically malignant appearing cells.

In the adolescent age group Amerson⁸ reported that cystosarcoma phyllodes occurs in about 5% of cases and concluded that these lesions are usually benign and are treated by extensive local excision rather than by loss of the adolescent breast.

Kier et al.⁹ indicated that giant fibroadenoma and cystosarcoma are closely related variants of a similar pathologic process. They concluded that a lack of a certain estrogen antagonist, such as progesterone, may

be a significant causative factor in the development of benign breast lesions, not merely excessive estrogen stimulation. Wulsin, ¹⁰ in a discussion of large breast tumors in young females, claimed that fibroadenomas, virginal breast hypertrophy, and cystosarcoma phyllodes are all related to each other by a common etiology of excessive estrogen stimulation. However, Oberman¹¹ in 1979 emphasized an abnormality of end-organ response and cited cases in which the estrogen levels were normal.

Giant fibroadenomas of youth are often encountered in young black girls after the onset of menstruation; however, these huge breast tumors have been observed in adolescent girls in the prepubertal period. 12-16 It is emphasized that the estrogen stimulation of the ordinary fibroadenoma may be enhanced in the giant fibroadenoma of the adolescents by an increased local sensitivity to elevated estrogen in combination with the absence of progesterone.

In the literature there is a difference of opinion regarding the surgical management of giant fibroadenomas of the breast. McDonald and Harrington in 1950 emphasized that these breast tumors are potentially malignant and should be considered so until proven otherwise. Another consideration with a bearing on the treatment was that these tumors involve practically the entire breast. The surgical treatment recommended by these authors was a simple mastectomy, not local excision of the tumor.

Hines and Guerkink¹⁷ indicated that these rare giantsize breast tumors in adolescent female patients may create a problem in management and that the rapid growth, distended veins, and varied histologic findings have often led to difficulties in diagnosis, and in many instances the surgical procedures have been unnecessarily radical. Ashikari et al.18 made a distinction between the more common adenofibroadenoma and the variant of this type, namely, the juvenile fibroadenoma. They described this latter tumor as usually solitary, quite large, varying in size from 2.5 to 19 cm, slightly firm, round or oval, freely movable, and encapsulated. When the lesion is quite large, they suggested that the tumor can be easily removed through a curved incision at the lateral or inferior border of the breast, and that one should avoid a mastectomy even if the tumor is large. Others state that excision is proper treatment,10,15,16 and Haagensen5 adds that irradiation and hormonal treatment provide no benefit.

In conclusion, the surgical treatment of any one of these huge enlargements of the breast should be individualized, and the extent of the surgical excision must depend on the pathologic conditions found at operation. Surgical removal of these large benign breast tumors is accomplished in most instances through a curved, inframammary incision that leaves adjacent normal breast tissue and the nipple-areolar complex, if possible, for reconstruction. The pathologist must examine many blocks of tissue to make certain no focus of malignant tumor cells is overlooked. The surgeon is urged to encourage patients to return for repeated examinations over a period of years because of possible development of recurrent tumors, whether benign or malignant. Haagensen⁵ points out that, in any one patient, recurrent fibroadenomas may develop as long as any breast tissue remains.

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Risk factors for complete molar pregnancy from a case-control study

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Demographic, reproductive, and dietary histories for 90 white women with complete molar pregnancy were compared in a multivariate analysis with those of 90 parous controls matched to cases by residence, birth year, and race. Women with molar pregnancy were more likely to have been born outside North America (relative risk = 1.9, p = 0.05), were more likely to have been past age 30 at time of their molar pregnancy (relative risk = 1.6, p = 0.05), and were more likely to have diets deficient in the vitamin A precursor carotene. Women with dietary scores for carotene above the control median had a relative risk for molar pregnancy of 0.6 (p = 0.02). In addition, there was a significant trend for decreasing risk for molar pregnancy with increasing consumption of carotene. Although other nutritional deficiencies in patients with complete molar pregnancy may exist, carotene is a biologically plausible candidate for a nutritional risk factor that could explain the geographic distribution of molar pregnancy. (AM J OBSTET GYNECOL 1985; 152:1016-20.)

Key words: Molar pregnancy, epidemiology, diet, carotene, fat

Description of the epidemiologic features of molar pregnancy has advanced little beyond the definition of broad demographic risk factors such as age, race, and geographic origin. Essentially no personal risk factors, such as contraceptive experiences or dietary habits, have been defined. Thus we undertook a case-control study of molar pregnancy and present the findings in this article.

Methods

The cases were white women with complete molar pregnancy referred to the New England Trophoblastic Disease Center for evaluation and therapy between 1975 and 1980 and interviewed between December, 1981, and June 1982. Only patients with a confirmed histologic diagnosis of complete molar pregnancy who were residents of Massachusetts were chosen for study. Out of 150 potential cases, 32 (21%) had moved from the state, 15 (10%) could not be reached by phone or letter, and three (2%) declined to participate. Another 10 patients were interviewed but could not be matched

to control subjects because they lived in an area of Massachusetts without Town Books (see below); they were excluded in this analysis based on matched pairs.

Characteristics of the molar pregnancy patients who could not be interviewed or matched were similar to those of the interviewed cases (about 55% in both groups had had a pregnancy with a live birth prior to the molar pregnancy). Cases that could not be reached because they had moved from their last known address were younger than those who were reached. However, this was also true for the controls that had moved.

The controls were selected from the Massachusetts Town Books, annual publications that list residents by name, age, and address. The controls matched the cases by year of birth (within 2 years), race, and precinct of residence, as a marker for socioeconomic status. It was also required that controls must have been delivered of a live-born infant. Of 187 women approached by introductory letter and telephone call, 44 (23%) had moved from that address, 14 (7%) could not be reached, 20 (11%) had not had a live-born infant, 15 (8%) declined to participate, and four (2%) were ineligible because of race or language. These exclusions left 90 case-control pairs as the basis for analysis.

Telephone interviews were conducted to assess a variety of factors including demographic history, pregnancies, contraception, prior gynecologic surgical procedures, smoking, coffee use, and dietary preferences. Exposure information such as contraceptive use, gynecologic surgical procedures, prior pregnancies, and smoking history was censored after the date of an index pregnancy defined for cases and controls. The index

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Table I. Distribution of case-control pairs and associated risk for molar pregnancy for various exposures, classified dichotomously

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Factor	Present in both	Absent in both	Present in cabsent in con		Present in control, absent in case	Relative risk*	p Value
Subject's age ≥30 at index pregnancy	9	56	20		. 5	4.0	0.005
Spouse's age ≥30 at index pregnancy	19	37	24		10	2.4	0.03
Subject's birthplace outside North America	0	77	11		2	5.5	0.03
Spouse's birthplace outside North America	1	80	9		0 (2)	11.1†	0.008
Catholic religion	42	12	16		20	0.8	0.6
Age ≥14 at menarche	-5	47	24		14	1.7	0.1
Oral contraceptive failure in index pregnancy	0	83	4		3	1.3	1.0
Spermicide failure in index pregnancy	0	80	6		4	1.5	0.8
IUD failure in index preg- nancy	0	88	1		1	1.0	1.0
Smoking during or prior to index pregnancy	9	39	20		22	0.9	0.9
Induced abortion prior to index pregnancy	0	81	8		1	8.0	0.05
Oral contraceptive use prior to index pregnancy	38	15	25		~ 12	2.1	0.05
Spermicide use prior to in- dex pregnancy	9	48	19		14	1.4	0.8
IUD use prior to index pregnancy	* 1	73	12		4	3.0	0.08
Protein intake above control median	15	24	21		30	0.7	0.3
Animal fat intake above control median	17	32	13		28	0.5	0.03
Carotene intake above con- trol median	17	34	11		28	0.4	0.02

See text for definition of index pregnancy.

pregnancy was defined as the first molar pregnancy for the case and as the pregnancy with the same sequence number for the matched control. This method of censoring the exposure information in the controls is necessary because the occurrence of the molar pregnancy predated our interview by several years in some instances. Other methods of censoring the exposure information such as using the calendar date of the case's molar pregnancy in the matched control would not have allowed age at pregnancy to be studied as an "exposure" variable since case and control were already matched on year of birth.

Each subject was asked about general diet including use of whole milk, cheese, butter, eggs, cabbage, cauliflower, brussel sprouts or broccoli, carrots, leafy greens, chicken, turkey, processed meats (hot dogs, salami, bologna, sausage), beef, pork, lamb, fish, regular coffee, decaffeinated coffee, tea, sodas or tonic, wine, beer or liquor. The frequency of intake was categorized as daily use, weekly use, or never or less than weekly use. The consumption of coffee, tea, and sodas was measured in cups or cans per day. Crude intake scores for various nutrients were calculated based upon the subject's frequency of use of food items times the nutrient content of a typical portion. The median value for any particular nutrient was identified for the control population, and members of each case-control pair were classified as "exposed" on the basis of whether their score fell above or below that control median.

A paired analysis is appropriate since we matched for age, race, and residence. The effect of an exposure, controlling only for the matching factors, was tested with McNemar's³ procedure based on dichotomous pairs. The effect of an exposure, controlling for other confounders, was examined with the use of multivariate logistic regression for paired data as described by Bres-

^{*}The relative risk is adjusted for the matching variables (year of birth, race, and residence).

[†]Unpaired analysis used to estimate crude exposure odds ratio.

Table II. Matched multivariate model with greatest predictive value of risk for molar pregnancy*

Factor	Adjusted relative risk†	95% Confidence limit	p Value
Carotene consumption above control median	0.6	0.4-0.9	0.02
Subject's birthplace outside North America	1.9	1.0-3.6	0.05
Subject's age ≥30 at index pregnancy	1.6	1.0-2.4	0.05
IUD use prior to index pregnancy	1.7	0.9-3.1	0.10

^{*} χ^2 Statistic to test all coefficients = 0 is 13.46 (4 df, p = 0.009).

low et al. Finally, the presence of a dose response for a particular variable was tested by a matched pair technique for three exposure levels developed by Pike et al. 5

Results

The average age at interview for cases was 31.9 years and for controls 32.2 years. The index pregnancy (first molar pregnancy) for cases was the first pregnancy in 29 (32%) instances, the second pregnancy in 20 (22%) instances, and the third or later pregnancy in 41 (46%) instances. Forty-eight (53%) subjects had had a liveborn pregnancy before their molar pregnancy. No instances of twin pregnancies prior to or subsequent to the molar pregnancy occurred in this series. In all but one case, the index pregnancy prompted the first referral to the New England Trophoblastic Disease Center. Two of the ninety cases have had a subsequent molar pregnancy and one a subsequent postterm choriocarcinoma.

Table I shows the distribution of case-control pairs and associated risk for molar pregnancy for various exposures, classified dichotomously. Exposures found to be associated with a significantly increased risk (p ≤ 0.05) for molar pregnancy included subject's or spouse's age ≥30 years at index pregnancy, subject's or spouse's birthplace outside North America, induced abortion, and use of oral contraceptives prior to the index pregnancy. Exposures found to be associated with a significantly decreased risk ($p \le 0.05$) for molar pregnancy included animal fat intake above the control median and carotene intake above the control median. Exposures found to be associated with an increased risk for molar pregnancy of borderline statistical significance (p = 0.1) included age ≥14 years at menarche and use of the intrauterine contraceptive device (IUD) prior to the index pregnancy. Exposures notable for their lack of association with molar pregnancy included religion, prior spermicide use, contraceptive failure during the index pregnancy, smoking prior to or during the index pregnancy, and protein intake above the control median. Other exposures found not to be significantly related to molar pregnancy but not reported in Table I include: consanguinity, use of prescription medications (especially analgesics for dysmenorrhea) prior to the index pregnancy, regular use of alcohol, coffee consumption or other caffeinated beverage use, medical illness or reproductive losses prior to the index pregnancy, gynecologic surgical procedures prior to the index pregnancy, and occupation (professional, clerical, service, or factory).

Some variables indicated to be significant in Table I are interrelated. To examine confounding among these variables, multivariate logistic regression was performed. Beginning with all the variables listed in Table I, step-down regression was performed until the β-coefficient of each remaining variable had a statistical probability of ≤10% of being equal to zero. This yielded the four-parameter model shown in Table II. Carotene consumption above the control median was associated with a relative risk of 0.6 (p = 0.02) and 95% confidence limits of 0.4 to 0.9 adjusted for age at index pregnancy, birthplace, and IUD use. Subject's birthplace outside North America was associated with an adjusted risk of 1.9 (p = 0.05) and confidence limits of 1.0 to 3.6. Subject's age ≥30 years at index pregnancy was associated with an adjusted risk of 1.6 (p = 0.05) and 95% confidence limits of 1.0 to 2.4. Use of the IUD prior to the index pregnancy was associated with an adjusted risk of 1.7 which, although not statistically significant, was included because it yielded the multivariate model which best accounted for the data. Spouse's age and spouse's birthplace were interchangeable with subject's age and birthplace in the model without materially affecting risks associated with carotene consumption.

Distributions of carotene intake scores are shown in Table IIIA, and trend in risk associated with level of carotene consumption is examined in Table IIIB. A significant trend (p = 0.01) for decreasing risk for mo-

[†]The relative risk for any factor is adjusted for the remaining three factors as well as for the matching variables (year of birth, race, residence).

Table IIIA. Distribution of carotene intake scores in case-control pairs

	Distribution in controls						
Distribution in cases	Lowest third	Middle third	Upper third				
Lowest third	15	11	18				
Middle third	10	13,	7				
Upper third	5	6	5				

Table IIIB. Associated trend of carotene intake in relative risk for molar pregnancy

Category of carotene consumption*	Relative risk†	95% Confidence limit
Lowest third	1	-
Middle third	0.6	0.2-1.2
Upper third	0.4	0.1-0.8

^{*}Based on thirty-third and sixty-sixth percentile intake scores for controls, the Mantel extension χ for linear trend is -2.49 (p = 0.01).

lar pregnancy was associated with increasing carotene consumption such that women in the upper third for carotene consumption had about one third the risk for molar pregnancy of women in the lowest third.

Distributions of animal fat intake scores are shown in Table IVA. Although the significant association between low dietary animal fat and molar pregnancy identified in the simple paired analysis did not persist in the multivariate analysis, it is of interest that there was a significant trend (Table IVB). Women in the upper third for animal fat consumption had about one third the risk for molar pregnancy of women in the lowest third (Table IVB).

Comment

Geographic origin and age appear to be important determinants of the frequency of hydatidiform mole and other forms of trophoblastic disease. The incidence is low in the United States⁶ and Europe⁷ and greater in Asia.⁸ Increasing age at pregnancy is also associated with increased risk for hydatidiform mole,^{6,7} although some studies have also suggested greater rates in adolescents.⁹ Virtually all of the studies cited are based on hospital or registry data in which the demographic profile of patients diagnosed as having a molar pregnancy is compared with that of women delivered of a live-born infant. Such studies are limited in that they do not permit the identification of personal risk factors

Table IVA. Distribution of animal fat intake scores in case-control pairs

	Distribution in controls						
Distribution in cases	Lowest third	Middle third	Upper third				
Lowest third	15	14	13				
Middle third	8	9	7				
Upper third	5	7	12				

Table IVB. Associated trend of animal fat intake in relative risk for molar pregnancy

Category of animal fat consumption*	Relative risk†		i i i	95% Confidence limit
Lowest third Middle third	1 0.9	. 46		0.3-2.0
Upper third	0.4			0.2-0.9

^{*}Based on thirty-third and sixty-sixth percentile intake scores for controls, the Mantel extension χ for linear trend is -2.12 (p = 0.03).

among individuals with similar demographic characteristics.

To meet this deficiency we performed a case-control study based on personal interview of 90 white women with complete hydatidiform mole and 90 parous controls matched to cases by birth date, race, and residence. Our findings concerning birthplace and age confirm the studies cited above. Women born outside North America had a 1.9-fold increased risk for molar pregnancy as compared to those born in North America. Spouse's birthplace was also significantly related to risk for molar pregnancy. In our analysis of age as a risk factor for molar pregnancy, we controlled for parity by comparing age at the first molar pregnancy with age at the pregnancy of the same sequence number in the age-matched control. Women pregnant past the age of 30 had a 1.6-fold increased risk for having a molar pregnancy compared to those pregnant before age 30. Spouse's age at index pregnancy was also a risk factor for molar pregnancy but not as strong as maternal age.

Pregnancy at an older age may more likely be preceded by contraception or abortion and this may confound the association between prior oral contraceptive use or abortion and molar pregnancy found in a simple paired analysis of the data in Table I. In a multivariate analysis adjusted for age at pregnancy, induced abortion and oral contraceptive use did not persist as statistically significant risk factors. It should also be con-

[†]The relative risk is adjusted only for the matching variables.

[†]The relative risk is adjusted only for the matching variables.

sidered that there may be preferential recall of such exposures by cases. However, it is noteworthy that contraceptive failure of the IUD, oral contraceptives, or spermicides in the index pregnancy was not associated with increased risk in this small study.

Geographic differences in the incidence of hydatidiform mole have been attributed to nutritional factors with protein deficiency cited as a possible factor. Our study of diet in relation to molar pregnancy compared intake scores for protein, fat, and carotene for cases and controls. Although no differences were noted between cases and controls for protein intake scores, cases differed from controls in animal fat and carotene consumption with the difference favoring a protective effect for these nutrients. Carotene consumption was the variable of greatest predictive value for molar pregnancy risk in the multivariate analysis of the data.

It should be emphasized that the food question naire used in this study was not exhaustive in quantifying all nutrients. In particular, the omission of breads, cereals, fruits, and liver from the questionnaire did not permit scoring of total carbohydrates, water-soluble vitamins, and preformed vitamin A. Since levels of one nutrient are often correlated with levels of other nutrients, the deficiencies in carotene and animal fat consumption observed for cases may simply correlate with deficiency of other nutrients. Furthermore the validity of these dietary data in relation to molar pregnancy depends upon the assumption that dietary patterns in individuals are relatively constant over time. Nevertheless, the suggested associations between molar pregnance and deficiency of carotene (and possibly fat) do have biologic credibility.

Both carotene, as a precursor to vitamin A, and fat have effects on reproduction. Vitamin A deficiency in the male rat and rhesus monkey produced degeneration of the seminiferous epithelium with consequent production of primitive spermatogonia and spermatocytes, while deficiency in the female rat and mesus monkey produced fetal resorption or abortion. ¹⁰⁻¹³ Deficiency of fat is also associated with degeneration of the seminiferous epithelium in male rats and fetal resorption and impaired ovulation in female rats and mice. ¹²⁻¹³ However, the influence of dietary fat deficiency on reproduction could be mediated in part through its effect on carotene absorption. When the diet is deficient in fat, the absorption of carotene is limited. ¹⁴

Geographic areas with a high incidence of vitamin A deficiency¹⁵ correspond to regions with a high incidence of molar pregnancy. Carotene and vitamin A deficiency have general effects on epithelial differentiation in addition to their specific effects on reproduction already

discussed. Epidemiologic evidence linking deficiency of carotene and human cancer has recently been reviewed. However, it is not clear precisely how deficiency of fat or carotene could cause molar pregnancy or how they might relate to the androgenetic origin of hydatidiform moles.

Given the exploratory nature of this study it is premature to conclude there is a causal relationship between molar pregnancy and carotene or fat deficiency, but our findings do indicate the need for further studies of nutrition and molar pregnancy with emphasis on carotene or other fat-soluble vitamins and animal fat.

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Morphologic characteristics of the placenta in glycogen storage disease type II (α -1,4-glucosidase deficiency)

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Five placentas from infants with enzymatically diagnosed glycogen storage disease type II (three from midtrimester abortions, two from term deliveries) were studied by light and electron microscopy. On routine histologic examination with hematoxylin and eosin staining, storage cells were identifiable in the connective tissue of the amnion. These cells provide the means to diagnose this glycogen storage disease prior to the development of clinical symptoms. Electron microscopy, even in the midtrimester placenta, shows typical membrane-bound, glycogen-filled vacuoles in the villous endothelium and stromal cells. These vacuoles can provide confirmation of glycogen storage in cases of prenatal enzymatic diagnosis and therapeutic abortion. (Am J Obstet Gynecol. 1985;152:1021-6.)

Key words: Placenta, glycogen storage disease type II, Pompe's disease, I-cell disease

Glycogen storage disease type II is defined by a deficiency of α-1,4-glucosidase, a lysosomal glycogen hydrolase. The morphologic features of tissue examined by electron microscopy in this disease are analogous to those of other lysosomal storage diseases in that the noncatabolizable substrate (glycogen in this case) remains in and fills the lysosomes. When these storage lysosomes become large and numerous, they can be detected by light microscopy. In glycogen storage disease type II the placental morphologic features have not been reported in detail. The placental morphologic characteristics of other lysosomal storage diseases, for instance, I-cell disease,2.3 GM1-gangliosidosis,4 mucolipidosis type IV,5 and Hurler's disease,6 have been reported. In these diseases, the pathologic findings were diagnostically useful.

Material and methods

After the autopsy in Case 1 at our hospital, placental tissue was obtained from the hospital of birth. The remaining cases of glycogen storage disease type II were from a collection of Epon-embedded cubes of tissue of approximately 1 mm saved from cases referred to the diagnostic enzymology laboratory. Control cases were selected from placentas processed in Epon in the pathology department as a routine part of other studies. All cases are listed in Table 1. The history of Case 1 is presented in detail below.

A formalin-fixed, paraffin-embedded block of pla-

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centa from Case 1 was routinely processed and stained with hematoxylin and eosin and periodic acid—Schiff with and without diastase. All Epon blocks were processed routinely and stained with a modified periodic acid—Schiff technique.⁷ Selected sections were cut for electron microscopy, routinely stained with uranyl acetate and lead citrate, and viewed with a Phillips 300 electron microscope.

Case report

This 1-year-old black girl had been delivered by cesarean section because of third-trimester bleeding from placenta previa. She weighed 5 pounds, 11 ounces and required no unusual resuscitation or hospitalization. At 8 weeks of age, she was admitted to her local children's hospital because of quivering movements. She had normal skull x-ray films and head computerized tomographic scan. The liver enzymes were noted to be elevated. At 11 weeks of age she was readmitted and had normal studies of protein loading with serum ammonias, α₁-antitrypsin electrophoresis, TORCH titers, and electroencephalogram. At 4 months of age, she was readmitted and had a liver biopsy done which was read as an unknown type of storage disease. At 6 months of age she was readmitted because of increasing shortness of breath and lethargy. She had radiographic, electrocardiographic, and echocardiographic evidence of massive bilateral ventricular enlargement and a 2 cm increase in liver size in 2 months.

She was then referred to Cincinnati Children's Medical Center where the diagnosis of glycogen storage disease type II was confirmed enzymatically and morphologically on liver biopsy. She was enrolled in therapeutic trials of theophylline and later carnitine without any therapeutic benefit. Despite digoxin and furosemide therapy, she died of congestive heart failure at 1 year of age.

Postmortem examination showed the typical findings

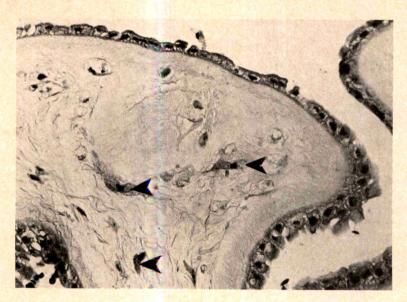


Fig. 1. The *arrows* point to the storage cells in the connective tissue of the amnion in Case 1. (Hematoxylin and eosin stain. Original magnification $\times 500$.)

Table I. List of cases and findings

Case no. D	2 3 1	Gestational age	Lysosomal glycogen by electron microscopy	Periodic acid-Schiff positive material on light microscopy					
	Diagnosis			Syncytio- trophoblast	Langhan's cells	Stromal cells	Vessel wall		
1	GSD II	Term	Yes	+	+	+++	+++		
2	GSD II	Term	Yes	Rare	+	+++	+++		
3	GSD II	Second trimester	Yes	0	+	+++	+++		
4	GSD II	Second trimester	Yes	Rare	+	+++	+++		
5	GSD II	Second trimester	Yes	Only chorion, stromal cells were positive					
6	I-cell disease	Second trimester	Yes	+	+	+	+		
7	Trisomy 21	Second trimester	No	0	0	+	0		
8	Turner syndrome	Second trimester	No	Only membranes, stroma, and vessels were positive					
9	V.AB.	First trimester	No	0	0	++	+		
10	V.AB.	9 wk	No	0	++	+	+		
11	V.AB.	Second trimester	No	0	+	++	+		

GSD II = Glycogen storage disease type II; V.AB = voluntary abortion. 0, +, ++, +++: Semiquantitative evaluation of the amount of periodic acid–Schiff positive staining.

of glycogen storage disease type II. She had massive cardiomegaly (185 gm, 5.6% glycogen), massive hepatomegaly (302 gm, 9.7% glycogen), and bulky skeletal muscle (8.3% to 13.5% glycogen). She had pericardial and bilateral pleural effusions. There was atelectasis of the lower lobe of the left lung from cardiac compression of the bronchus. Microscopically, besides liver and striated muscle, glycogen deposition was especially prominent in neurons, smooth muscle, and ova. Scattered skeletal muscle fibers show pools of alcian bluepositive, basophilic material similar to that reported in the literature.¹

Results

Vacuolated storage cells in the connective tissue of the amnion were a distinctive finding in Case I (Fig. 1). These vacuoles were relatively uniform in size with sharp borders, which distinguished them from the foamy changes often seen in amniotic stromal cells. The vacuoles stained with periodic acid—Schiff before but not after diastase digestion. In other placental mesenchymal cells the high normal content of cytoplasmic glycogen made the distinction of normal from glycogen storage disease type II unreliable even with the periodic acid—Schiff stain.

The findings in the remaining cases are shown in Table I. Neither the pattern of periodic acid-Schiff staining nor the distribution showed a reliable distinction between glycogen storage disease type II and a normal placenta with the use of light microscopy. Glycogen in cases and controls was distributed in a punctate

pattern in Langhan's cells and villous stromal cells and vessel walls (Fig. 2). By electron microscopy, all placentas in cases of glycogen storage disease type II showed membrane-bound vacuoles full of glycogen in the distribution seen by light microscopy (Figs. 3 and 4). The only control to show similar glycogen vacuoles was the case of I-cell disease where endothelial cells showed similar vacuoles by electron microscopy (Fig. 5). Other cells in this case showed numerous, large, heterogeneous vacuoles diagnostic of I-cell disease.

Diastase digestion was not possible after osmium fixation and Epon embedding; however, the electron microscopic localization coincided with the granular periodic acid-Schiff positivity in cases and controls.

Comment

The results of this study have value in three different contexts. The findings in Case 1 suggest that the diagnosis of glycogen storage disease type II can be made prospectively in a fortuitously examined placenta. The diagnosis would be difficult without some suspicion, but if a child presented with early, nondiagnostic symptoms, reexamination of the placenta should be diagnostic. The amniotic storage cells can be identified and histochemically shown to contain glycogen. This approach would have saved diagnostic time and expense in Case 1.

The second context is in the confirmation of the prenatal diagnosis of glycogen storage disease type II made by enzyme assay. The electron micrographic findings in the second trimester show the pathognomonic accumulation of lysosomal glycogen. Inclusion cell disease does show accumulation of lysosomal glycogen; however, there is no diagnostic problem as this disease is characterized by more numerous lysosomes with varied debris. First-trimester villi in normal conceptions show cytoplasmic glycogen in the same cells that show lysosomal storage in glycogen storage disease type II.8 We are optimistic that the electron microscopic identification of lysosomal glycogen will allow rapid diagnosis of glycogen storage disease type II by transcervical chorionic biopsy in the first trimester of pregnancy.

The last context is the use of glycogen storage disease type II as a probe of the role of lysosomal glycogen metabolism in the placenta and in cells in general. The usual pathogenetic explanation of glycogen storage disease type II is that lysosomal glycogen accumulates as an incidental cytoplasmic component during autophagocytosis. The role of lysosomal salvage of glucose is considered negligible in the cell economy, but the clearing of glycogen from the lysosome is vital to lysosomal, and eventually to cell, function. However, Hug9 has demonstrated that this interpretation is inadequate to explain the correlations of morphologic



Fig. 2. Villi from Case 2 showing dark black material positive for periodic acid-Schiff in the stroma cells (arrowhead), fetal vessels (V), and Langhan's cells (arrow). (Epon embedded. Periodic acid-Schiff stain. Original magnification ×840.)

features and dysfunction in patients with glycogen storage disease type II. The findings in the placenta show that lysosomal glycogen accumulates in cells already rich in cytoplasmic glycogen but is conspicuously absent from syncytiotrophoblast, which has scant cytoplasmic glycogen. This distribution of storage vacuoles supports a passive mechanism of glycogen accumulation.

The I-cell disease case requires further comment. Icell disease results from a defect in the mannose-6phosphate receptor system, which routes lysosomal enzymes. Adequate α-1,4-glucosidase activity would be expected to be absent from lysosomes, as would other lysosomal hydrolases, in I-cell disease. Thus the glycogen-filled lysosomes in the endothelial cells of the Icell placenta lend support to the concept that the glycogen accumulation in glycogen storage disease type II is solely the result of the absent enzyme activity. A similar phenomenon has been seen in the liver in rats given an inhibitor of α-1,4-glucosidase.10 The dominance of glycogen in the placental endothelial cell inclusions is not explained.

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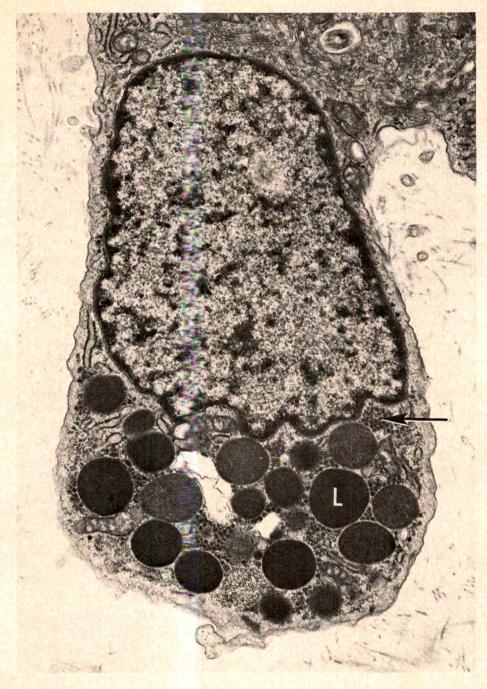


Fig. 3. Electron micrograph of a strongal cell from Case 2 showing membrane-bound glycogen (L) as well as unbound cytoplasmic glycogen rosettes (arrow). (Lead citrate and uranyl acetate \times 12,880.)

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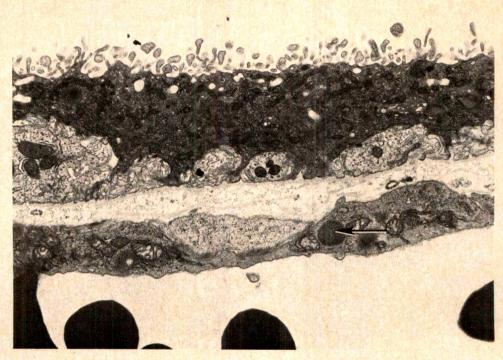


Fig. 4. Electron micrograph. In Case 2, the syncytiotrophoblast at the top does not contain membranebound glycogen. The fetal endothelial cell does have membrane-bound glycogen (arrow). (Lead citrate and uranyl acetate. Original magnification ×11,700.)

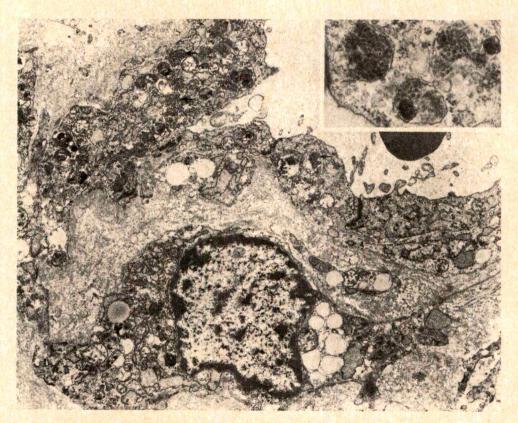


Fig. 5. Electron micrograph. In the I-cell disease placenta, the endothelial cells contain apparent glycogen rosettes in membrane-bound inclusions (arrows and inset). The stromal cell at the bottom exemplifies the heterogeneous inclusions of this disease. (Lead citrate and uranyl acetate. Original magnification ×14,720. Inset: Original magnification ×40,500.)

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Evaluation of the pharmacodynamics and pharmacokinetics of ritodrine when administered as a loading dose

On establishing a potentially useful drug administration regimen in cases of fetal distress

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Inhibition of labor during the intrapartum period has been suggested as a method of managing acute fetal distress. In such cases, rapid tocolysis is desirable but, in high doses, β-adrenergic-receptor agonists, such as ritodrine, may cause severe maternal hypotension that could aggravate the existing fetal distress. We undertook the present study to establish a safe infusion protocol for ritodrine that achieves high plasma concentration rapidly. Twelve nonpregnant female volunteers received, on separate days, three infusions of ritodrine, that is, 1, 2, and 3 mg, during a 2-minute period. The peak plasma concentration measured by high-performance liquid chromatography with electrochemical detection averaged 37, 74, and 100 ng/ml after the 1, 2, and 3 mg doses, respectively. Fitodrine concentrations decreased rapidly and with the 3 mg dose the ritodrine concentration was only 14 ng/ml after 15 minutes. The elimination phase half-life of ritodrine averaged 6.11 hours. None of the doses significantly affected systolic blood pressure but ritodrine increased heart rate and the plasma glucose level and decreased diastolic blood pressure and the plasma potassium concentration. Even at the highest infusion rate, the maximal changes in cardiovascular and metabolic variables were short-lived and clinically modest; heart rate increased 29 bpm, diastolic blood pressure decreased 8 mm Hg, glucose level increased 26 mg/dl, and potassium concentration decreased 0.6 mEq/L. These data indicate that high plasma concentrations of ritodrine can be achieved rapidly without serious side effects. (AM-J OBSTET GYNECOL 1985;152:1026-31.)

Key words: Ritodrine, pharmacodynamics, pregnancy

Inhibition of labor during the intrapartum period has been suggested as a method of managing acute fetal distress. ¹⁻⁴ β-Adrenergic—receptor stimulants are the agents used most often, although magnesium sulfate has also been successfully used. ⁵ This approach to intrapartum fetal distress has been utilized for many years, ¹⁻³ but it has not been widely accepted in the United States. A few reports from this country have been published recently²⁻⁴⁻⁵ but the use of socolytic

agents for fetal distress is still considered to be experimental.

There is no established regimen of drug administration for ritodrine in cases of intrapartum fetal distress. The drug has been given as a bolus, as an infusion, or as a combination of both. ^{1,6,7} The amount of ritodrine given has been empirical and has not been based on kinetic properties of the drug. In cases of acute intrapartum asphyxia, rapid tocolysis is desirable so that uterine activity is abolished as quickly as possible. ¹ However, β-adrenergic—receptor agonists in high doses may cause severe maternal hypotension that could aggravate the existing fetal distress. ^{8,9} We undertook the present study as a preliminary step in establishing a safe infusion protocol for rapid intrapartum tocolysis. For ethical reasons we chose to study nonpregnant female volunteers. Our objectives in the current study were two-

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Table I. Effect of ritodrine on heart rate, systolic and diastolic blood pressures, and plasma potassium and glucose concentrations in nonpregnant women

			Dose	
		0.5 mg/min	1.0 mg/min	1.5 mg/mîn
Heart rate (bpm)				
Control		77 ± 4	79 ± 4	73 ± 3
2 min		$87 \pm 2*$	$98 \pm 4*$	102 ± 4*
5 min		$87 \pm 3*$	94 ± 4*	91 ± 4*
 Systolic blood pressu 	ire (mm Hg)			
Control	,	106 ± 2	103 ± 3	103 ± 3
2 min		108 ± 4	105 ± 3	108 ± 3
5 min		106 ± 3	104 ± 3	106 ± 3
Diastolic pressure (n	nm Hg)			
Control		64 ± 2	65 ± 2	66 ± 2
2 min		$60 \pm 2*$	$61 \pm 2*$	58 ± 3*
5 min		60 ± 2*	57 ± 3*	58 ± 3*
Potassium (mEq/L)				
Control		4.2 ± 0.1	4.2 ± 0.1	4.2 ± 0.1
30 min		$3.6 \pm 0.1^*$	$3.6 \pm 0.1*$	$3.6 \pm 0.2*$
120 min		$3.9 \pm 0.1*$	$3.8 \pm 0.1^*$	$3.7 \pm 0.1*$
Glucose (mg/dl)				
Control		87 ± 4	87 ± 4	87 ± 4
30 min		$113 \pm 13*$	$131 \pm 13*$	113 ± 8*
120 min		99 ± 5	94 ± 4	104 ± 4

Duration of infusion was 2 minutes. Values are mean ± SE.

Table II. Pharmacokinetic parameters of ritodrine in nonpregnant women

			Volume of distribution		
Distribution phase half-life (hr)	Elimination phase half-life (hr)	Central (L/kg)	Apparent (L/kg)	Steady state (L/kg)	Plasma clearance (L/kg/hr)
0.032 ± 0.004	6.11 ± 0.57	0.26 ± 0.03	4.19 ± 0.12	3.62 ± 0.18	0.52 ± 0.06

Values are mean \pm SE; n = 10. Based on an infusion of 1.5 mg/min for 2 minutes.

fold. First, we wanted to monitor the systemic effects produced by ritodrine with an infusion regimen that achieves high plasma concentrations rapidly. Our second objective was to define the pharmacokinetic behavior of ritodrine in nonpregnant women by the method of high-performance liquid chromatography with electrochemical detection.10 Data describing the pharmacokinetics of ritodrine in pregnant or nonpregnant women are very limited.11, 12 Gandar et al. 12 used a radioimmunoassay to measure ritodrine in six nonpregnant women but reported only the mean values of a few kinetic parameters.

Material and methods

This study was approved by the Human Experimentation Committee of Magee-Womens Hospital. Informed consent was obtained from 12 nonpregnant female volunteers, all of whom were within 10% of ideal body weight and between the ages of 18 and 45. We excluded women with hypertension, diabetes, cardiac disease, liver or renal disease, or central nervous system disorders. Women who were treated with other sympathomimetic agents, methylxanthines, narcotics, parasympatholytics, or potassium-depleting drugs were also excluded. Prior to the study, each subject underwent a complete physical examination; and an electrocardiogram was obtained. Each one had blood withdrawn for the determination of a complete blood count with differential, platelet count, urinalysis, and levels of serum glutamic oxaloacetic transaminase, serum glutamic pyruvic transaminase, alkaline phosphatase, bilirubin, blood urea nitrogen, creatinine, glucose, and potassium. Acceptance into the study required a normal physical examination, clinically acceptable laboratory values, and a negative urine pregnancy test.

Each volunteer received a ritodrine infusion of 0.5, 1.0 and 1.5 mg/min for a 2-minute period (a total of 1, 2, or 3 mg, respectively). For each volunteer, a minimum of 2 days elapsed between any two studies. During each study, an indwelling venous catheter was in-

^{*}Value significantly (p < 0.05) different from control.

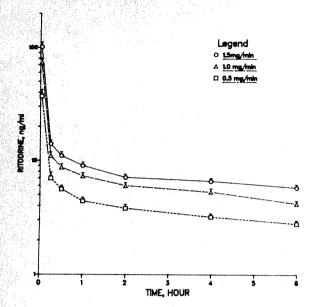


Fig. 1. Plasma concentration of ritodrine in nonpregnant women following a 2-minute infusion of ritodrine at a rate of either 1.5, 1.0, or 0.5 mg/min. Values are mean and SE (n = 11).

serted into each arm. The subject was in the dorsal recumbent position with the head of the bed elevated 30 to 45 degrees. Prior to administering ritocrine, we infused 400 ml of 5% dextrose in water during a 20to 30-minute period. We recorded the electrocardiogram (lead I) continuously from 1 minute prior to the initiation of the ritodrine infusion until 30 minutes after initiation of the infusion. Blood pressure was recorded periodically with a sphygmomanometer. Ritodrine, 1, 2, or 3 mg, was diluted in a volume of 18.75 ml of 5% dextrose in water. Three blood pressure and heart rate measurements were obtained at 5-minute intervals prior to initiation of the infusion of raodrine; these values served as control. Ritodrine was infused for a 2-minute period by a constant-infusion pump. Blood pressure and heart rate were obtained at 1, 2, 5, 10, 20, and 30 minutes from the start of the infusion. Blood was withdrawn from the indwelling catheter prior to and at 2, 5, 15, 30, 60, 120, 240, and 360 minutes after initiation of the ritodrine infusion. The blood was collected in heparinized tubes and centrifuged, and the supernatant was aspirated. Some of this plasma was frozen and later used to measure the concentration of ritodrine by the method of high-performance liquid chromatography with electrochemical detection.10 Plasma from the 30- and 120-minute samples was analyzed within 3 hours for glucose and potassium concentrations. Thirty minutes after the infusion was completed, the subjects were allowed to sit in a chair for an additional 30 minutes for observation. Thereafter, they were allowed up at will. During and immediately after the infusion, the subjects were questioned regarding side effects such as chest pain, shortness of breath, nausea, or palpitations.

Data analysis

For each volunteer, plasma concentration data with the 3 mg dose were fitted to a two-compartment model with use of the NONLIN Program Package. 13 Correlation coefficients (r) between actual and predicted data averaged 0.96 ± 0.01 (SE). The NONLIN program provided the necessary constants that enabled us to perform simulations of drug concentration during various infusion rates. Changes in cardiovascular variables and glucose concentration were compared by repeated measures analysis of variance with dosage level and time as the two within-group factors.14 Furthermore, we also compared the differences in cardiovascular variables and glucose concentrations between the control and posttreatment time periods by the method of multiple comparisons with the use of the Satterthwaite approximation for the degrees of freedom.14 We used the paired t test to compare pretreatment and posttreatment concentrations of potassium because if repeated measures analysis of variance was used, too many subjects would be dropped out of the analysis because of missing data.

Results

The effect of ritodrine infusion on several cardiovascular and metabolic variables is summarized in Table I. With all doses, the peak heart rate was observed at the end of the 2-minute infusion. Statistically significant changes in heart rate occurred with all doses, but they were of greater magnitude and longer duration with the higher dose. Heart rate rapidly returned toward pretreatment values, and 30 minutes after the highest dose, the heart rate was only 11% above baseline. Systolic blood pressure was not significantly affected by ritodrine even at the highest infusion rate (Table I). A statistically significant decrease in diastolic blood pressure was noted with all doses but only at 2 and 5 minutes after the start of the infusion. The maximal reductions in diastolic blood pressure, however, were only 4, 8, and 8 mm Hg less than control with the 1, 2, and 3 mg doses, respectively.

Table I also shows the effect of ritodrine on plasma glucose and potassium concentrations 30 and 120 minutes after the start of the ritodrine infusion. The potassium level decreased and the glucose level increased significantly at 30 minutes. At the 120-minute sample, concentrations had begun to return toward control values.

None of the volunteers reported serious side effects. All noted some palpitations but none had chest pain,

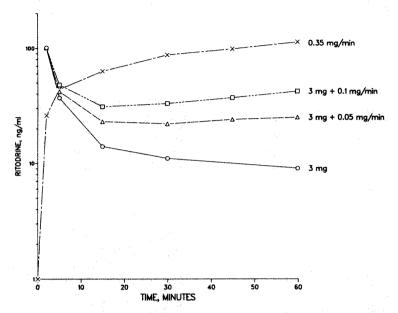


Fig. 2. Plasma concentration of ritodrine in nonpregnant women during various infusion regimens. Infusions of 0.35, 3 mg \pm 0.10, and 3 \pm 0.05 mg/min are simulated. The 0.35 mg/min infusion began at time 0 and lasted 60 minutes; the remaining three infusions began with a 2-minute infusion of 1.5 mg/min and were followed with either 0.10 or 0.05 mg/min or nothing for the remaining 58 minutes.

shortness of breath, or vomiting. The continuously recorded electrocardiogram demonstrated minor nonspecific ST- and T-wave changes in some subjects but these changes were not diagnostic of ischemia.*

Fig. 1 depicts the plasma ritodrine concentration following the three infusion rates. Ritodrine could not be measured in one woman because of an interfering substance in the plasma so she was excluded from those analyses relating to ritodrine concentration. The disappearance of ritodrine from plasma was initially very rapid, indicating a rapid distribution to various tissues. Even with the 3 mg dose, the concentration of ritodrine remained above 20 ng/ml for only about 12 minutes. After the initial rapid distribution phase, ritodrine was eliminated rather slowly. We used the raw data from the 1.5 mg/min infusion to calculate pharmacokinetic parameters of ritodrine. These parameters are summarized in Table II. One of the 11 women was excluded from this analysis because two data points were missing.

Fig. 2 shows the ritodrine plasma concentration during a 60-minute period during four different infusion regimens. The first three curves (0.35 mg/min for 60 minutes, 3 mg during 2 minutes followed immediately by either a 0.10 or a 0.05 mg/min infusion for the remaining 58 minutes) are simulations based on the constants determined by the NONLIN program and

*Interpretations done by James Kirshenbaum, M.D., and Elliott Antman, M.D., Brigham and Women's Hospital, Department of Medicine, Cardiovascular Division.

the pharmacokinetic parameters in Table II. The fourth curve (3 mg during 2 minutes with no supplemental infusion) is taken from Fig. 1. Peak concentration with the 3 mg loading dose was 100 ng/ml whereas with the 0.35 mg/min infusion, 45 minutes was required to reach this concentration. The ritodrine concentrations were comparable at about 5 minutes when, with all infusion regimens, the concentration was 35 to 45 ng/ml.

Comment

The concentration of ritodrine required to inhibit labor is not clearly established, but in a recent study we found that a concentration of 15 to 45 ng/ml was sufficient to inhibit labor in a majority of cases.11 One of the objectives of the present study was to determine if infusion rates that rapidly achieve high serum concentrations of ritodrine are well tolerated. We used infusion rates that produce plasma concentrations similar to those that might be effective in pregnant women. Our data indicate that in nonpregnant women high serum concentrations of ritodrine can be achieved rapidly without serious side effects. Ritodrine infused at a rate of 1.5 mg/min for 2 minutes increased the heart rate (40%) and plasma glucose level (30%) and decreased diastolic blood pressure (12%) and the plasma potassium level (14%). These changes were statistically significant but are clinically modest and are comparable to changes observed when ritodrine is given to women in preterm labor. We have previously reported that

ritodrine treatment of women with preterm labor and intact membranes increases heart rate and glucose level by an average of 35% and 60%, respectively, while diastolic blood pressure and potassium level are decreased by 11% and 27%, respectively.15

Using pharmacokinetic principles, we were able to simulate plasma ritodrine concentrations during various infusion regimens. Drug concentration versus time curves differ considerably when ritodrine is given in the conventional manner as opposed to the rapid loading dose we have described here. With high affusion rates of short duration, high plasma concentrations are achieved rapidly. With the highest currently approved infusion rate of 0.35 mg/min, plasma concentration rises more slowly. Theoretically, in cases of fetal distress, labor inhibition should be achieved as rapidly as possible without causing maternal hypotension or other serious maternal side effects. The higher infusion rates of short duration may achieve this objective. If continued labor inhibition is required, the initial infusion can be supplemented with an infusion of 0.05 to \$35 mg/ min as required. The pharmacologic principles presented will also apply to pregnant women although there probably will be quantitative differences in drug kinetics¹⁶ and perhaps cardiovascular responsiseness.

Based on limited data in the literature. 6-8 it is likely that a ritodrine infusion of 1.5 mg/min for a 2-minute period will be as well tolerated by pregnant women as it is in nonpregnant women. Sheybany et al.6 gave ritodrine at a rate of 2.0 mg/min for 3 minutes (6 mg total) to 24 pregnant women in order to treat fetal Estress. Hypotension reportedly did not occur but blood pressure data were not reported in the paper. Uterine activity was reduced by 78% during the 15-minute interval following the drug infusion and fetal condition apparently improved. Lipshitz et al. administered a 6 mg bolus of ritodrine to pregnant women whose labors were being induced at term with oxytocin. Feak cardiovascular changes with this large bolus dose included a 55% increase in heart rate and a 42% decrease in diastolic pressure. The oxytocin-induced uterme activity was reduced by 43% in the first 10 minutes. Even with this large dose of ritodrine, Lipshitz et al did not report any adverse fetal effects. Hutchon gave a 2 mg bolus of ritodrine to four pregnant women with severe fetal bradycardia. No serious side effects were reported but limited maternal cardiovascular data were presented. The fetal heart rate recovered in all cases.

We measured ritodrine by the method of high-performance liquid chromatography with electrochemical detection.10 Our data are the first that describe the pharmacokinetic behavior of ritodrine in nonpregnant women with the use of this method. We fitted our data to a two-compartment model. The data points

predicted by our biexponential equations correlated strongly with the actual data. The ritodrine concentration decreases rapidly at the end of infusion, indicating a large volume of distribution. After this phase, the disappearance of ritodrine from plasma is slower, the elimination phase half-life being 6.1 hours. Gandar et al.12 measured ritodrine with a radioimmunoassay and fitted their data to a three-compartment model. Our kinetic data differ considerably from those of Gandar et al. Pharmacokinetic studies by radioimmunoassay and high-performance liquid chromatography with electrochemical detection are required to compare the two assay methods.

In the present study, we have demonstrated that high plasma ritodrine concentrations can be achieved rapidly without serious side effects. Future studies can evaluate the tolerance to ritodrine by pregnant women receiving infusions similar to those described in the present study. Based on the cardiovascular responses in nonpregnant volunteers in this study and the few studies in pregnant women described in the literature, 6-8 it is likely that such regimens will be tolerated well.

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The effect of oral ritodrine therapy on glucose tolerance in pregnancy

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Intravenous ritodrine therapy can cause significant deterioration of maternal glucose homeostasis. We investigated the effect of full maintenance oral ritodrine therapy (120 mg/day) on glucose tolerance in the early third trimester with the use of 50 gm 1-hour screens followed by 100 gm 3-hour oral glucose tolerance tests if the screen level was ≥140 mg/dl. Four hundred ninety-one patients were studied, 42 of whom were receiving oral ritodrine therapy. Twenty-one percent of the ritodrine-treated women had an abnormal 1-hour screen, which was not different from the 20% observed in women not receiving therapy. None of the treated group and 13% of the untreated group who had abnormal screens had abnormal oral glucose tolerance tests. The probability of an abnormal test after an abnormal 1-hour screen was also determined. (AM J OBSTET GYNECOL 1985;152:1031-3.)

Key words: Pregnancy, oral ritodrine therapy, glucose tolerance

Intravenous ritodrine therapy can cause significant deterioration of maternal glucose homeostasis. ^{1, 2} The effects of maintenance oral ritodrine have not been well studied. Because patients in preterm labor may require long-term treatment with oral ritodrine at the time of greatest insulin resistance, 26 to 30 weeks' gestation, the metabolic consequences of such treatment could be of critical importance. This study was undertaken to determine the effects of oral ritodrine therapy on carbohydrate tolerance in the early third trimester.

Subjects and methods

The study included all patients in our high-risk practice between September, 1980, and March, 1984. A 1-hour 50 gm oral glucose screening test was performed

routinely at 27 to 32 weeks' gestation. (89% of all patients were tested between 28 and 30 weeks' gestation). All patients on ritodrine had received at least 72 hours of therapy prior to testing. Seventy-four percent had received at least 1 week of oral therapy and 50% more than 2 weeks of oral therapy prior to testing. The full standard dose of ritodrine, 120 mg/day (10 mg every 2 hours), was used. Patients were excluded from the study if they were known to have diabetes, had a history of gestational diabetes, were receiving other β-sympathomimetic agents, or were exposed to corticosteroids within 72 hours of testing. Women treated with ritodrine and untreated women were comparable with respect to age (30.7 versus 31.1 years), obesity, chronic hypertension, and family history of diabetes (Table I). Only 7% of the treated and 5% of the untreated patients were less than 25 years of age.

Blood was obtained from all subjects by venipuncture 1 hour following a 50 gm oral glucose load. Patients did not necessarily fast prior to testing. Ritodrine therapy was continued throughout glucose tolerance testing without change in dosing interval. Glucose determinations were performed on serum samples by the glucose oxidase method. A 1-hour serum glucose value of >140 mg/dl was considered abnormal and prompted

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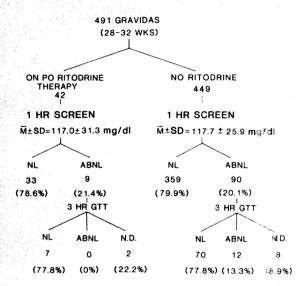


Fig. 1. Results of 1-hour serum glucose screening in 491 patients. N.D. = Not done.

Table I. Group comparability

	Oral ritodrine	No ræadrîne
No.	42	449
$Age \pm SD (yr)$	30.7 ± 4.8	31.1 ± 4.2
Prepregnancy weight ± SD (pounds)	126.2 ± 26.5	135.1 ± 26.8
Family history of diabetes* (%)	14.3	12.0
Hypertension† (%)	2.3	3.6

There are no statistically significant differences between groups.

*First-degree relatives only.

†History of hypertension whether currently treated or not.

further evaluation with a 100 gm oral glucose tolerance test. The diagnosis of gestational diabetes was made when two or more abnormal values were observed during the 3-hour oral glucose tolerance test. A fasting value \geq 105 mg/dl, a 1-hour value \geq 190 mg/dl, a 2-hour value of \geq 165 mg/dl, and a 3-hour value \geq 145 mg/dl were considered abnormal as recommended by the National Diabetes Group in 1979.7 Results were analyzed by Student's t test and χ^2 analysis as applicable.

Results

Four hundred ninety-one patients were studied, 42 of whom were receiving oral ritodrine therapy (Fig. 1). Of this group, 21.4% had an abnormal 1-hour value, an incidence similar to the 20.1% observed in patients who were not receiving ritodrine. None of the patients with abnormal screening values in the ritodrine-treated group were found to have gestational diabetes. Thirteen percent of the untreated group did demonstrate two or more abnormal values on the oral glucose tolerance test. The mean 1-hour screening glucose values

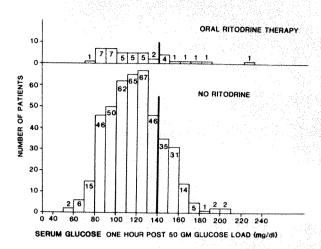


Fig. 2. Histogram of 1-hour serum glucose results for patients receiving oral ritodrine and those not receiving ritodrine.

were 117.0 ± 31.3 mg/dl for ritodrine-treated patients and 117.7 ± 25.9 mg/dl for the control group. As shown in Fig. 2, there was no unusual distribution of glucose values in either group. The probability of an abnormal 3-hour oral glucose tolerance test after an abnormal 1-hour screening test was also determined (Table II). Only five of 65 patients (7.7%) with screening values between 140 and 160 mg/dl had abnormal 3-hour tests. Almost 30% of women with 1-hour values between 160 and 180 mg/dl developed gestational diabetes. Of six patients with screening values >180 mg/dl, two had abnormal 3-hour tests.

Comment

Although oral ritodrine therapy is widely used in the second and third trimesters of pregnancy, scant attention has been directed to its effect on maternal glucose tolerance. Only two reports have addressed this question. They include a total of 18 patients receiving less than the currently recommended 120 mg daily dose of ritodrine. Blouin et al.8 performed a 3-hour oral glucose tolerance test after a 50 gm oral glucose load in eight women receiving 40 mg/day of ritodrine daily and six control subjects at 25 and 35 weeks' gestation. They found no significant differences in the test results in these two groups. Schreyer et al.9 studied 10 pregnant women prior to and immediately following a 10-day course of 80 mg of oral ritodrine daily. Using an intravenous glucose tolerance test, they demonstrated no differences in glucose tolerance in these patients before or after therapy.

Our study includes a larger number of treated patients receiving the recommended 120 mg/day dose of ritodrine. We have observed no significant change in glucose tolerance in women maintained on a regimen of a full therapeutic dose of oral ritodrine as compared to similar women who were not treated.

Table II. Positive predictive value of abnormal 1-hour screening tests

		Current study	Carpenter and Coustan ¹¹		
Serum glucose 1 hour after 50 gm load (mg/dl)	n n	Abnormal glucose tolerance test (%)	n	Abnormal glucose tolerance test (%)	
130-139 140-159 160-179 180-199 200-220	65 18 3 3	7.7 27.8 33.3 33.3	33 53 12 6 2	9.0 16.9 33.3 83.3 100	

These results differ from the abnormal glucose homeostasis that may occur acutely during intravenous ritodrine infusion. This difference may be drug level related as oral therapy results in significantly lower blood levels than does intravenous therapy. An alternative explanation is that the alteration in carbohydrate tolerance induced by ritodrine may be an acute effect that resolves spontaneously during several days. Hastwell and Lambert10 demonstrated an increase in the serum glucose level in six patients (two at 29 to 32 weeks' gestation, four at 14 to 16 weeks' gestation) treated with oral salbutamol during the first hours of therapy. Glucose levels returned to baseline values by 72 to 96 hours. All of our patients were tested after at least 72 hours of treatment. It is also possible that the patients for whom oral ritodrine was prescribed may not have taken the medication. We believe that this is very unlikely as our private patient population is highly motivated. Furthermore, we monitored maternal pulse rates in patients on a regimen of oral ritodrine therapy and found an appropriate increase in nearly all cases. In addition, 20 of the 42 patients receiving ritodrine were tested as inpatients and their results did not differ from those of patients who were tested as outpatients.

The number of positive 1-hour screening values in our population, 99 of 491 or 20.2%, is similar to the 21% positive rate observed in the original study of O'Sullivan et al.⁴ of unselected women who were ≥25 years of age. Fourteen percent (12 of 89) of our patients and 19% of O'Sullivan's original subjects had abnormal follow-up 3-hour oral glucose tolerance tests.

The positive predictive values of abnormal 1-hour tests in our population differ from those reported by Carpenter and Coustan.¹¹ These differences are summarized in Table II. One possible explanation for these differences may be that our patients did not receive carbohydrate loading or fast prior to testing. However, if this were the case one would expect many more positive screens in our population. Twenty percent of our patients and 19.1% (73 of 381) of Carpenter and Coustan's patients had 1-hour screening values ≥140 mg/dl. These authors did use slightly different criteria for the diagnosis of gestational diabetes after a 3-hour oral glucose tolerance test. However, applying their test cri-

teria to our data redefines only two patients as having gestational diabetes. Both were in the control group, one with a 1-hour value of 143 mg/dl and one with a value of 196 mg/dl. The most plausible explanation is that sample size, particularly in the markedly abnormal ranges, is small in both studies and may lead to discrepancies in the positive predictive values. It appears, however, that many patients with significantly abnormal 1-hour screens may have a normal 3-hour oral glucose tolerance test.

In summary, full-dose oral ritodrine therapy does not appear to affect glucose tolerance in nondiabetic patients. Additionally, the 1-hour 50 gm glucose loading test is a useful screening technique, although its positive predictive value when ≥180 mg/dl may not be as high as previously claimed.

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Luteinizing hormone-releasing hormone agonist and uterine leiomyoma: A pilot study

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Ten women with 12 uterine leiomyomas ranging from 7.5 to 420 cc (mean, 112.6 ± 39.4) were treated with subcutaneous injections of the luteinizing hormone—releasing hormone agonist buserelin, 200 μg three times daily for 1 week and then 500 μg daily for the rest of the 6-month treatment period. Following initial stimulation the pituitary ovarian axis was suppressed after 3 weeks of treatment with mean serum estradiol ranging between 17 and 36 pg/ml. Seven uterine leiomyomas had a marked regression in size following treatment with luteinizing hormone—releasing hormone agonist; two were undetectable and the volume of the other five diminished by an average of 80%. One tumor did not respond to treatment, two regressed by 25%, and two, following an initial reduction of 65% and 50%, reenlarged during the last 2 months of treatment to 75% and 100% respectively of their initial volume. Luteinizing hormone—releasing hormone agonist is the first medication demonstrated effective in reducing the size of uterine myomas. (AM J OBSTET GYNECOL 1985;152:1034-8.)

Key words: Luteinizing hormone-releasing hormone, uterine leiomyoma, pituitary desensitization

The classical treatment of a woman with symptomatic uterine leiomyoma has until now been surgery. Depending on the need to preserve the fertility potential and the size of the tumor, hysterectomy or selective surgical myomectomy was offered.

The interest in treating uterine leiomyoma with luteinizing hormone–releasing hormone agonist stems from the fact that, as we have reported for endometriosis, this medication induces a state of reversible hypogonadism without intrinsic steroidal effect. Herein we report a pilot study on 10 patients with uterine leiomyoma treated with luteinizing hormone–releasing hormone agonist.

Material and methods

Subjects. Ten women, aged 27 to 51 years (mean 34.6 ± 2.3), were included in the study after giving informed consent. Six patients were nulligravid, and four had a previous pregnancy. Indications of treatment were abdominal pain or pelvic pressure (five women), infertility with otherwise normal workup (three women), habitual abortion (one woman), and

recent progression (one woman). All of the uterine leiomyomas were positively identified by gynecologic examination and by ultrasound. In six patients a laparoscopic confirmatory diagnosis was also available. Eight patients had one leiomyoma whereas two patients presented with two leiomyomas. Initial volume of these uterine tumors ranged from 7.5 to 420 ml (mean, 112.6 ± 39.4).

Protocol. Before treatment, baseline information was determined, including complete medical history, physical examination, and safety laboratory investigation (complete hemogram, urine analysis, SMA-16, triiodothyronine, thyroxine, cortisol, testosterone, prolactin, pulmonary x-rays, x-rays of the fine bones of the hands, electrocardiogram). Treatment was started on days 2 to 5 of the cycle. During the first 7 days, 200 µg of buserelin ([D-Ser(TBU)6-des-gly-NH210] LHRH ethylamide, Hoechst Canada Inc., Montreal, Canada) was administered subcutaneously every 8 hours, followed by 500 µg subcutaneously daily for the rest of the 6month treatment period. Acute response to luteinizing hormone-releasing hormone agonist administration has been monitored on the first day of treatment and after 1, 4, 8 weeks, and 3 and 6 months of buserelin administration. Ultrasonographic measurement of the leiomyomas was carried out every month by the same technician and physician. Complete physical examination and safety laboratory tests were performed after 3 and 6 months of treatment. Statistical analysis was done by the Duncan-Kramer method.

Laboratory methods. Serum luteinizing hormone

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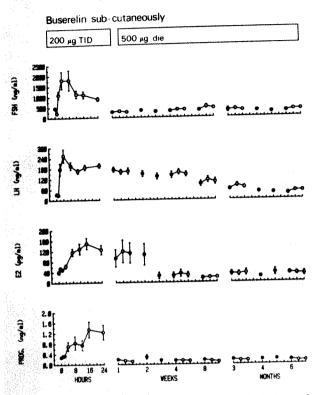


Fig. 1. Mean \pm SEM of serum FSH, LH, estradiol (E_2), and progesterone (PROG.) levels before and during buserelin treatment. Closed circles indicate basal hormonal levels, whereas open circles indicate hormonal response 4 and 8 hours after drug administration. During the first day of treatment the detailed 24-hour response is illustrated.

and follicle-stimulating hormone were measured by specific radioimmunoassays.2 Materials for luteinizing hormone and follicle-stimulating hormones were supplied by the National Pituitary Agency, National Institutes of Health, Bethesda, Maryland. The results are expressed in nanograms per millimiter of LER-907. Early follicular phase values range from 85 to 400 ng/ ml for follicle-stimulating hormone and from 25 to 60 ng/ml for luteinizing hormone.

Estradiol and progesterone were measured by highly specific sera and tritiated tracers. Duplicate serum aliquots were extracted with anhydrous ether, and activated charcoal was used to separate free and antibodybound steroids. Assay sensitivity was 5 to 10 pg/ml for estradiol and 0.1 to 0.2 ng/ml for progesterone.

Results

Serum gonadotropin and sex steroid levels. Basal levels of serum gonadotropins and sex steroids as well as their acute response to luteinizing hormone-releasing hormone agonist administration are shown in Fig. 1. Before beginning the treatment the mean basal levels of luteinizing hormone and follicle-stimulating hormone were 33.8 ± 4.3 ng/ml and 465.9 ± 48.7 ng/ml,

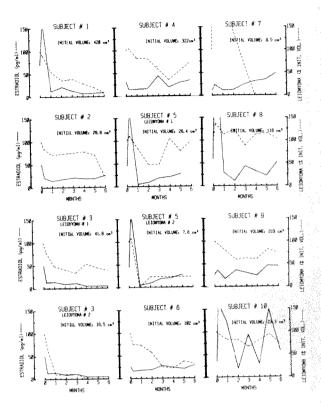


Fig. 2. Individual curve of volume of uterine leiomyomas (dotted line) and serum estradiol levels (solid line). The volume of uterine leiomyoma is expressed in percentage of the initial volume.

respectively. Following the first subcutaneous injection of 200 µg of buserelin, maximum 7.5- and 3.8-fold increases of serum luteinizing and follicle-stimulating hormones were observed. After 1 week of treatment, serum follicle-stimulating hormone levels were already suppressed whereas serum luteinizing hormone levels still slowly decreased thereafter.

Before treatment the mean serum estradiol levels were 40 ± 5.6 pg/ml. Following gonadotropin stimulation, the mean serum estradiol levels increased to a maximum of 149.1 ± 21 pg/ml at 16 hours. Mean serum estradiol value progressively decreased thereafter and stabilized 3 weeks after initial injection at levels between 17 \pm 2.53 and 36 \pm 13.1 pg/ml for the rest of the treatment period. One exception is Patient No. 10 in whom serum estradiol levels were not suppressed by luteinizing hormone-releasing hormone agonist treatment and oscillated during the last 5 months of treatment between 19 and 149 pg/ml.

After 1 week of treatment, luteinizing hormone-releasing hormone agonist administration did not elicit any statistically significant acute elevation of serum gonadotropin or sex steroids levels. This lack of response of gonadetropins and gonadal steroids to luteinizing hormone-releasing hormone agonist administration persisted for the rest of the treatment period.

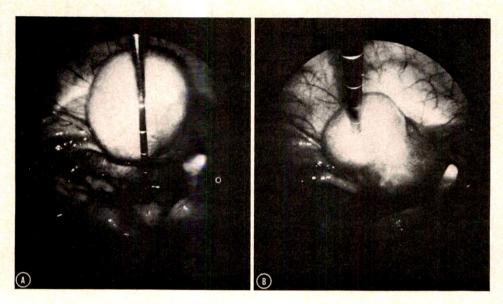


Fig. 3. Pelvic endoscopic photographs of Patient No. 6 before (A) and after (B) treatment with luteinizing hormone–releasing hormone agonist. A 68% volume reduction in the 182 cc initial volume of uterine leiomyoma is illustrated.

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Fig. 4. Pattern of hot flushes during treatment with buserelin.

Volume of uterine leiomyomas. Individual response of uterine leiomyomas and serum estradiol levels before and during treatment with luteinizing hormone–releasing hormone agonist treatment are presented in Fig. 2. A total of 12 uterine leiomyomas were observed. Seven uterine leiomyomas presented a marked regression following treatment with luteinizing hormone–releasing hormone agonist; two at the end of treatment were undetectable by ultrasound and gynecologic ex-

amination and the volume of the other five had diminished by an average of 80%. One tumor (No. 8) did not respond to treatment, two (No. 9 and No. 10) regressed by 25%, and two (No. 4 and No. 5-1) had an initial reduction of 65% and 50% but reenlarged during the last 2 months of treatment to respectively 75% and 100% of their initial volume. Fig. 3 consists of pelvic endoscopic photographs of patient No. 6 taken before (A) and after (B) treatment with luteinizing hormone–releasing hormone agonist. A 68% reduction in the 182 ml initial volume of uterine leiomyoma is illustrated

Side effects and safety laboratory data. As shown in Fig. 4, hot flushes were the most common side effect, usually starting 3 to 4 weeks after initiation of treatment. Eight patients reported a diminution of vaginal secretions and nine experienced dryness of the mouth. Two patients developed transitory dyspareunia during treatment. However, libido and orgasm were not affected by the treatment.

The pattern of vaginal bleeding is illustrated in Fig. 5. Initial menstruation was prolonged in two patients (No. 2 and No. 9). Estrogen withdrawal bleeding was observed in 4 patients, in 3 after 1 month of treatment (No. 1, No. 5, and No. 7) and in one after 2 months of treatment (No. 10). Thereafter only occasional vaginal spotting occurred during the rest of the treatment period.

Slight but statistically significant (p < 0.05) elevation of serum glutamic-oxaloacetic transaminase, serum glutamic-pyruvic transaminase, alkaline phosphatase, and phosphorus as well as a reduction (p < 0.01) in concentration of neutrophils (56% compared to 65%

LEGEND Treatment Mild w3 W4 М2 M4 М5 W2 W1 patient Moderate KXXXX 01 Blood 02 Spotting 0000 03 04 XXX 05 06 07 08 09

Symptoms: Vaginal bleeding

Fig. 5. Pattern of vaginal bleeding during treatment with buserelin.

10

pretreatment level) were noted after 6 months of treatment. These biologic measurements, however, as well as the others listed in the material and methods section were within the normal range before and after 3 and 6 months of luteinizing hormone–releasing hormone agonist treatment. Treatment did not statistically affect any of the other biologic measurements.

Follow-up. Patient No. 4 presented with infertility, and because her uterine leiomyoma had reenlarged during the last months of treatment, selective surgical myomectomy was performed during the follow-up period. An edematous, histologically degenerated leiomyoma was removed. All the other patients have completed at least 3 months of follow-up. Normal menstrual bleeding rapidly returned (26 to 49 days after treatment), one exception being patient No. 7 in whom return of uterine bleeding took 3 months. Two leiomyomas (No. 6 and No. 10) continued to decrease slightly during the follow-up period, three remained stable (No. 5-2, No. 7, and No. 9), six reenlarged (No. 1, No. 2, No. 3-1, No. 3-2, No. 5-1, and No. 8) to an average of 73% of their initial volume. None of the patients who presented with habitual abortion or infertility is as yet pregnant.

Comment

Not all of the uterine leiomyomas responded to the hypoestrogenism induced by luteinizing hormone–releasing hormone agonist treatment. There was no correlation between the reduction obtained and the age of the patients or the initial volume of the tumor. The lack of response of some of the leiomyomas was expected, since some of these tumors after a while become composed almost exclusively of fibrous tissues and are no longer hormonally active.

Filicori et al.3 have reported a 77.5% reduction of

one uterine leiomyoma following 15 weeks of treatment with 8 µg/kg of D-Trp⁶-Pro⁹-NEt-LHRH administered daily by subcutaneous injection. A preliminary report of the first three patients described here has also been published.⁴

Luteinizing hormone-releasing hormone agonist is the first medication demonstrated as being effective in decreasing the size of uterine leiomyoma. In this pilot study, side effects were well tolerated, and all of the safety laboratory data stayed in the normal range.

We have reported⁵ a deceptive 20% reduction following use of danazol, 400 mg two times daily for 6 months. The number of cytoplasmic progesterone receptor binding sites in uterine leiomyoma is low and does not increase, as in normal myometrium, with the menstrual cycle.⁶ This may possibly explain why progestogens have not been demonstrated to be effective for these pathologic conditions.

However, prolonged hypoestrogenism is not without danger; it reduces the high-density lipoprotein cholesterol and may induce osteoporosis. Chronic treatment with luteinizing hormone–releasing hormone agonist should therefore be carefully evaluated before its clinical recommendation.

We wish to acknowledge the invaluable assistance of Marie Dandurand in the preparation of this manuscript.

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Platelets and uric acid in the prediction of preeclampsia

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Fifty-four pregnancies that were subsequently complicated by preeclampsia had platelet parameters and uric acid levels measured during pregnancy. The expected fall in platelet count and rise in platelet volume parameters and uric acid levels beyond those that occur in normal pregnancy were seen only in the week before delivery. (AM J OBSTET GYNECOL 1985;152:1038-9.)

Key words: Platelets, uric acid, preeclampsia

A fall in platelet count and a rise in serum uric acid levels are well-known features of preeclampsia. Hyperuricemia is said to be an early feature of prweclampsia, but there is dispute as to whether the coagulopathy and hence the fall in platelet count are early or late features of the disease.

Methods and material

Blood samples were obtained from women attending the antenatal clinics of Bristol Maternity Hospital during a 12-month period. A total of 2881 hematologic samples and 929 serum samples were obtained. Fifty-four of the patients from whom samples were obtained subsequently experienced preeclampsia. The peralts of the preeclamptic patients were grouped according to the time before delivery that the samples were obtained. These results were compared with the results from nonhypertensive pregnancies that were grouped according to time before 40 weeks that the samples were obtained. Preeclampsia is defined as hypertension with an elevation of the systolic blood pressure by 30 mm Hg or more or the diastolic pressure by 15 mm Hg or more with significant proteinuria (>0.3 gm/L) or, in the ab-

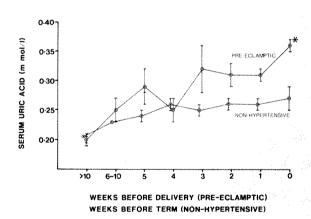


Fig. 1. Uric acid levels (mean \pm SEM) grouped in weeks before delivery for preeclamptic pregnancies and weeks before term for nonhypertensive pregnancies. *Asterisk* indicates significant difference (p < 0.01).

sence of proteinuria, a systolic blood pressure of 160 mm Hg or more or a diastolic blood pressure of 110 mm Hg or more. In the analysis of the platelet results, statistical significance was assessed by the Student *t* test. For the uric data, analysis of covariance was used to adjust for the different gestational ages of the two groups.

Platelets. The results from the nonhypertensive patients showed a fall in platelet count and rise in platelet volume parameters throughout pregnancy.² In pregnancies complicated by preeclampsia all three platelet parameters parallel the changes in normal pregnancy

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except in the week before delivery. In the final week of the preeclamptic pregnancies there is an appreciable fall in platelet count and rise in platelet volume parameters beyond those that occur in nonhypertensive pregnancies.

Uric acid. Results from nonhypertensive pregnancies showed a rising level continuously throughout pregnancy. In the preeclamptic pregnancies the uric acid values rose above the nonhypertensive values in the fourth week before delivery (Fig. 1) but differed significantly (p < 0.01) only in the week before delivery after adjustment was made for the gestational age.

Comment

Increased platelet destruction is associated not only with thrombocytopenia but also with macrothrombocytosis. Both of these features occur in preeclampsia but also to a lesser extent in normal pregnancy.² We found that changes in platelet number and volume in preeclamptic pregnancies parallel the changes in normal pregnancies and deviate from them, becoming more pronounced, only in the last week before delivery. These findings suggest that the increased platelet destruction and hence the coagulopathy are late phenomena of preeclampsia. The early fall in platelet count in preeclampsia as described by Redman et al.¹ could be explained if some of their preeclamptic patients were

delivered early in the third trimester, since this is when the difference between their preeclamptic and control patients became significant. The observed rise of uric acid levels throughout nonhypertensive pregnancy confirms previous reports. Redman et al¹ have suggested that patients who subsequently develop preeclampsia have significantly higher levels of uric acid from 28 weeks' gestation. Our findings suggest that uric acid rises significantly only in the week before delivery in patients who develop preeclampsia.

The results of any study of a parameter in pregnancy that alters with the development of preeclampsia cannot have the resultant values grouped and compared with the nonpreeclamptic control values on a gestational age (weeks of pregnancy) scale since preeclampsia can occur at any stage in the second half of pregnancy, and thus values from the test patients who develop this disease early may deviate the grouped test values. This study suggests that uric acid and platelet parameters are of little value in predicting preeclampsia.

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Sonographic sign for the detection of early fetal ascites in the management of severe isoimmune disease without intrauterine transfusion

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Ultrasonography is an important adjunct to the delta optical density at 450 nm in the management of isoimmunized pregnancies. We describe a sonographic finding that we believe is the earliest sign of fetal ascites suggesting decompensation. In this patient, despite high measurements of delta optical density at 450 nm, intrauterine therapy or delivery was delayed for more than 7 weeks by careful sonographic monitoring. (AM J OBSTET GYNECOL 1985;152:1039-41.)

Key words: Isoimmune disease, ascites, sonography

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Reprint requests: Beryl R. Benacerraf, M.D., Diagnostic Ultrasound Associates, 398B Brookline Ave., Boston, MA 02215. Isoimmune hemolytic disease of the fetus due to Rh blood group incompatibility is now a relatively rare disorder as a result of effective prevention. There remains, however, an occasional patient who is severely sensitized for whom refined diagnostic testing is nec-

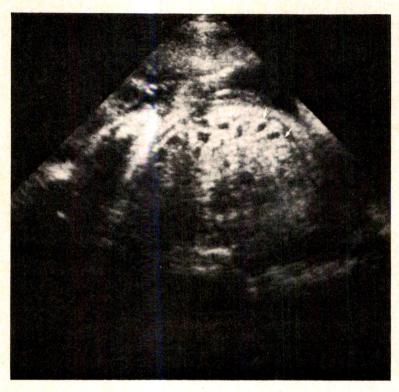


Fig. 1. Frame from real-time sonogram shows a cross section of the fetal abdomen with multiple bowel loops. Note that the bowel wall is well seen from either side (arrows).

essary to permit the most advantageous clinical decisions. Measuring serial amniotic fluid bilirubin by determination of the delta optical density at 450 nm (ΔOD_{450}) has been the benchmark of fetal assessment¹; however, more recently, fetal heart rate testing and sonographic assessment have evolved as important adjunctive indicators of fetal state.2 This report is an example of the refinements that are possible in ultrasonographic monitoring of the fetus at risk for the development of isoimmune hydrops. Although measuring amniotic fluid bilirubin as the ΔOD_{450} reflects the extent of hemolysis that has occurred, it gives limited insight into the degree of fetal compensatory mechanisms that may be accommodating to the hemolytic process. The ΔOD_{450} alone, therefore, is not sufficiently sensitive for the most effective clinical decision-making. We offer another refined sign that should help in the sonographic surveillance of this disease. We describe a case in which sonographic visualization of both sides of the bowel wall in a fetus at high risk for isoimmune hydrops was the key to identifying the earliest possible sign of ascites, indicative of severe decompensation long after the ΔOD_{450} would have indicated the need for intrauterine transfusion. This does not imply that the ΔOD₄₅₀ is not a necessary part of the clinical management of this problem. Rather it suggests that its use in conjunction with other laboratory modalities will increase clinical acumen.

Case report

M. G., a 30-year-old woman, gravida 6, abortions 2, para 2, therapeutic abortion 1, had a previous pregnancy that ended in intrauterine death hours after an intrauterine transfusion at 30 weeks. The present pregnancy was followed up from 11 weeks with serial ultrasound examinations and she underwent her first amniocentesis at 21 weeks for determination of the ΔOD_{450} . The fetus was observed sonographically on 20 separate occasions during the pregnancy and nine separate ΔOD₄₅₀ determinations were obtained. The results were in the high midzone and the last two were in the third zone. In the last week, sonographic observation was almost daily. During each of the early examinations, by the same sonographer, the ultrasound findings were entirely within normal limits, both anatomically and functionally, until 30 weeks. At that time, it was noted that both sides of the bowel wall in the fetal peritoneal cavity were visible, a finding that was interpreted as possible early ascites (Fig. 1). The biophysical profile was reassuring during this examination and the sonographic findings were unchanged the next day. Daily ultrasound examinations were performed, and 48 hours after the original observation a small but conventional rim of ascites was diagnosed.

When the initial unusual appearance of the bowel wall was noted, the mother was treated with glucocorticoids for immature pulmonic indices. When the ascites was confirmed, delivery of a 1654 gram live female infant with Apgar scores of 5 and 7 was accomplished, some hours after the last ultrasound examination. The

baby was grossly normal, without evidence of hydrops. The cord hematocrit was 20%. She required five exchange transfusions and ventilatory therapy for severe hyaline membrane disease during the first week of life. Her progress since that time has been uneventful.

Comment

Recently, specialists in maternal-fetal medicine have relied on ultrasonographic signs to help in the decisionmaking process when dealing with Rh-sensitized patients. Eight cases have been reported from this institution in which reassuring serial ultrasound examinations were used as the basis for avoiding intervention for 8 to 63 days despite high ΔOD₄₅₀ findings.²

These eight cases were managed without intrauterine transfusion despite ΔOD_{450} results in the third zone. The ultrasound appearance of the hydropic fetus is well known and includes ascites, scalp edema, and pericardial effusion as the salient features. A way of identifying the early signs of hydrops, however, would be crucial for this kind of optimal obstetric management as, once the full-blown hydropic features are present, the prognosis is severely reduced. Despite high $\Delta \mathrm{OD}_{450}$ findings in the previously reported cases and this report, very early detection of fetal decompensation was the deciding factor for intervention.

Visualization of both sides of the bowel wall is a wellknown radiologic sign on abdominal plain film for the diagnosis of free peritoneal air. When the same criterion is applied to a sonogram, visualization of both sides of the bowel wall indicates the presence of material (in this case, fluid) on the outside of the bowel wall, silhouetting it. This sign, which indicates a small amount of intraperitoneal fluid as in our case, preceded the identification of a frank rim of ascites by 2 days, thus facilitating obstetric management and enhancing fetal outcome. We suggest, therefore, that sonographic visualization of the outer aspect of the bowel wall in a fetus signifies small quantities of free fluid in the peritoneal cavity and that this may be one of the earliest signs of fetal hydrops and decompensation.

It should be noted that the traditional approach would have been to opt for intrauterine transfusion at 24 weeks on the basis of the previous history and three consecutive ΔOD_{450} results that were high in the midzone and rising. We suggest that the present combined approach of fetal assessment avoided the need for intrauterine therapy and other intervention for more than 7 weeks.

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The role of ultrasound in the aggressive management of obstructed labor secondary to fetal malformations

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Eleven cases of major congenital anomalies were diagnosed in conjunction with arrest of dilatation or descent. In three cases, the diagnosis of fetal death was made. In the remainder, despite extensive counseling, the mothers refused cesarean section for an anomalous fetus. Fetal decompression resulted in prompt vaginal delivery in 10 of 11 cases. (AM J OBSTET GYNECOL 1985;152:1042-4.)

Key words: Decompression, hydrocephalus, labor, prune-belly syndrome, ultrasound

Obstructed labor secondary to fetal malformations is an uncommon problem. Prior to the advent of modern techniques for cesarean section and the availability of intensive neonatal medical and surgical support, various destructive techniques that were commonly used were designed to avoid operative delivery even at the cost of fetal life. As the morbidity of cesarean section has fallen in past decades, fetal destruction for the sole purpose of averting abdominal delivery has become medically unjustifiable. Nevertheless, when confronted with the detailed description of a major congenital anomaly, some patients may be unwilling to undergo cesarean section for fetal salvage.

Methods

We report here our recent experience with 11 cases of major congenital anomalies, diagnosed intrapartum with ultrasound, in conjunction with arrest of dilatation or descent. In each case, the labor obstruction was felt to be secondary to the fetal malformation. In three cases, the diagnosis of fetal death was made; in the remaining eight, despite extensive counseling, the mothers refused cesarean section for an anomalous fetus. Fetal decompression was then accomplished with subsequent prompt vaginal delivery in 10 of 11 cases.

Results

Tables I, II, and III detail the clinical presentation, method of decompression, and autopsy data for these

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Reprint requests: Steven L. Clark, M.D., Department of Obstetrics and Gynecology, Los Angeles County/University of Southern California Medical Center, Women's Hospital, 1240 North Mission Road, Los Angeles, CA 90033. fetuses. In each case, postmortem examination confirmed the prenatal diagnosis suggested by ultrasound.

Comment

Congenital hydrocephalus may be of a minor degree and associated with a normal labor and vaginal delivery. However, quantities of cerebrospinal fluid as little as 280 ml may be associated with severe labor dystocia. Vaginal decompression via the anterior fontanelle seems to be the method of choice for hydrocephalic infants presenting in a vertex position. Once the diagnosis of gross hydrocephalus has been made, the decision for cesarean section or decompression should not be delayed, as uterine rupture with obstructed labor secondary to hydrocephalus has been reported well before full cervical dilatation.1 In cases of breech presentation, the physician should await spontaneous delivery of the fetal body and then proceed with decompression through the foramen magnum should spontaneous delivery not be possible. An alternative approach is transabdominal puncture under ultrasonic guidance. Obstructed labor secondary to the prune-belly syndrome may also be managed by transabdominal decompression. However, in some cases, spontaneous delivery of the fetal head with subsequent dystocia due to abdominal distention may mandate vaginal decompression.

When confronted with a major congenital anomaly preventing delivery, extreme caution must be exercised in counseling the parents regarding operative delivery. Although intrauterine measurement of cerebral cortical thickness is possible with ultrasound, the literature clearly demonstrates an inconsistent correlation between thickness of the cortical mantle and subsequent neurological outcome.² Thus, while some degree of neurological impairment may be anticipated, severe retardation is by no means certain. The prune-belly syndrome may be associated with megalocystis, hydroureter, renal dysgenesis, and other anomalies in addition to congenital absence of the abdominal wall mus-

Table I. Clinical data for hydrocephalic fetuses presenting in vertex position*

	Biparietal	Cortical	Fluid	Birth	Apgar	score	
Case No.	diameter (cm)	thickness (cm)	removed (ml)	weight (gm)	1 min	5 min	Associated anomalies
1	>14	1	Not stated	3860	0	0	Polymicrogyria, hypoplasia of medulla, agenesis of corpus callosum
9	11.1	Not stated	1500	3280	5	6	Dandy-Walker syndrome
2	10.4	1.3	350	2640	0	0	Autopsy refused
4	13.2	Not stated	1200		3	8	Bilateral hydronephrosis
5	11.3	"Minimal"	180	3840	0	0	Congenital absence of corpus callosum, stenosis of aqueduct of Sylvius

^{*}Decompressed vaginally.

Table II. Clinical data for hydrocephalic fetuses presenting in breech position*

	Biparietal	Cortical	Fluid	Birth	Apgar	r score	
Case No.	diameter (cm)	thickness (cm)	removed (ml)	weight (gm)	1 min	5 min	Associated anomalies
6	13.4	"Minimal"	500	4460	0	0	Arnold-Chiari malformation; limb anomalies
7	15.6	"None visible"	1400	2680	0.	0	Arnold-Chiari malformation; myelomeningocele
8	15.0	1.6	500	4200	0	0	Massive hydrops; multiorgan hemorrhage
9	14.5	Not stated	1655	2650	0	0	Absence of brain stem and cerebellum; myelo- meningocele

^{*}Decompressed vaginally (Cases 6 and 7) or transabdominally (Cases 8 and 9).

Table III. Clinical data for fetuses with prune-belly syndrome

· 			Birth	Fluid	Apgar	r score	
Case No.	Ultrasonic findings	Route of delivery	weight (gm)	removed (ml)	1 min	5 min	Autopsy findings
10	Massive fluid-filled abdomen	Vaginal after delivery of head	1300	1300	0	0	Prune-belly syndrome; bilateral renal dysgenesis; hypo- plastic lungs; bilat- eral clubfoot
11	Fluid-filled abdomen with 15 by 15 cm mass	Transabdominal	800	1500	0	0	Prune-belly syndrome; bilateral hydrone- phrosis; hypoplastic lungs; imperforate anus

culature. While advances in reconstructive surgery have resulted in satisfactory repair of the abdominal wall defect, ultimate prognosis is more closely related to the nature of the urologic anomalies. Thus this syndrome is not incompatible with life.

When faced with such anomalies, many parents will elect cesarean section, providing, from a fetal standpoint, the least traumatic and most desirable method of delivery. Should this be refused, the obstetrician is faced with the equally important responsibility of avoiding the serious maternal morbidity and mortality associated with protracted, obstructed labor. In such cases, decompression offers a safe and effective alternative. When faced with antepartum fetal death, this

approach may also allow the obstetrician to avoid cesarean section for a fetus with major anomalies precluding vaginal delivery.

The ethical and legal questions raised by the ability of ultrasound to detect congenital anomalies are complex. These problems are compounded for both parents and obstetrician when the diagnosis is first made in the face of obstructed labor, whereby an acute management decision is mandatory.

It seems reasonable to suggest that all hospital research and ethics committees should consider these problems in a nonemergent fashion so as to alleviate the significant burden placed upon the practicing obstetrician.

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Modification of fetal intraventricular amniotic shunt

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The benefits of fetal intraventricular amniotic shunt remain unknown. Nevertheless, the use of this technique has been described by several authors. One of the potential complications arising from its placement is the inability to retract the catheter once it is placed through the large needle. This communication describes a modification of the shunt procedure. A suture placed through the distal end of the catheter allows retrieval of the catheter shunt for as long as the needle remains in place. (AM J OBSTET GYNECOL 1985;152:1044-5.)

Key words: Fetal intraventricular amniotic shura, fetal hydrocephalus

The development of an intrauterine procedure for treatment of fetal hydrocephalus has seen limited success. A recent publication by Clewel et al. reported the successful implantation of a fetal intraventricular amniotic shunt in a 26-week fetus. The method described requires the unidirectional insertion of a catheter through the calvarium into the dilated fetal ventricle through a large-bore sheath under ultrasound guidance. This technique allows for limited control of the shunt. If the shunt is advanced too far into the head, it cannot be retrieved. In order to facilitate placement and retrieval of the shunt, we have modified the in-

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sertion technique as follows: A 6.0 (or smaller) Dexon suture is placed through an open hole in the proximal end (amniotic portion) of the shunt. Both ends of the suture are held as the shunt is inserted into the sheath as previously described (Fig. 1). The shunt is then advanced to the appropriate depth within the fetal head and removed according to the same procedure. If the shunt has been advanced too far, even beyond the sheath, it can then be removed with the aid of the suture tail.

Once proper placement has been identified, one end of the suture is pulled, thus the suture is retrieved.

Comment

In multiple insertions in an in vitro setting, we have yet to experience any difficulty in freely sliding the suture material back into the sheath. This includes instances in which the catheter has been passed completely beyond the needle. Although it is possible that

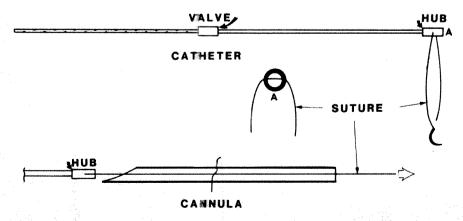


Fig. 1. Diagram of modified insertion technique.

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the suture may become twisted and its removal or passage made more difficult, we have yet to encounter this in an in vitro situation. If it occurs, we would recommend leaving the suture in utero since we see no significant added risk to the fetus. Although strangulation of a limb can theoretically occur, the risk should be minimal.

In summary, because of the increasing interest in fetal therapy, we have described a simple modification of the Clewel procedure for intracranial shunting. We believe that this added safety step will facilitate this technique should it prove to be beneficial to the fetus. Clearly, further study is warranted.

REFERENCE

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Neonatal effects of maternal nadolol therapy

Renee E. Fox, M.D., Celeste Marx, Pharm.D., and Ann R. Stark, M.D.

Boston, Massachusetts

Described is the case of an infant, exposed during gestation to the β-blocker nadolol, who experienced cardiorespiratory depression, mild hypoglycemia, and growth retardation. The longer duration of action of nadolol and the fact that it is only 30% protein bound make it less desirable than propranolol for use as a β-blocker during pregnancy. (AM J OBSTET GYNECOL 1985;152:1045-6.)

Key words: Infant, β-adrenergic agents, bradycardia, hypoglycemia, respiratory depression, growth retardation

Although the use of several β-adrenergic blocking agents has been reported in hypertension associated with pregnancy,1 no information is available about the effects on the fetus of the administration of nadolol. In this report, we describe the case of an infant whose mother received nadolol throughout gestation, and who developed problems associated with this drug.

Case report

L. B., a 1.8 kg male infant, was born after 35 weeks' gestation to a 37-year-old woman, gravida 2, para 0, who had an IgA nephropathy and hypertension. The mother's daily medications during pregnancy included nadolol (20 mg), Dyazide, and Armour Thyroid (3 grains). Her blood pressure was 135/90 mm Hg, and her creatinine clearance remained stable at 50 to 60 ml/min. Serial ultrasound examinations indicated poor fetal growth, and elective delivery was planned at 35 weeks since indices of lung development were mature. Although a nonstress test was reactive 18 hours before delivery, the fetal heart rate pattern became abnormal with a low baseline rate (110 to 120 bpm) and a brief late deceleration. The infant was delivered by emer-

From the Department of Pediatrics, Harvard Medical School, Brigham and Women's Hospital, and The Beth Israel Hospital. Received for publication August 24, 1984; revised December 21, 1984; accepted January 7, 1985. Reprint requests: Dr. Renee E. Fox, Joint Program in Neonatology,

75 Francis St., Boston, MA 02115.

gency cesarean section performed under epidural anesthesia. Apgar scores were 9 and 10 at 1 and 5 minutes, respectively. The mother's last dose of nadolol was 20 hours before delivery.

Findings on physical examination of the infant were normal, except for tachypnea to 68 breaths per minute. Heart rate was 136 bpm. Dubowitz examination was consistent with gestational age determined from menstrual history. Head circumference and length were fifteenth percentile, and weight was tenth percentile for 35 weeks' gestation. Hematocrit was 59%. Blood glucose, initially 20 mg/dl, rose to 40 mg/dl after feeding of glucose water.

At 41/2 hours of age, the infant's respirations slowed to 23 breaths per minute, heart rate decreased to 112 bpm, and temperature fell to 96.5° F. His blood pressure and color remained normal. Although warming normalized his temperature, the heart rate and respiratory rate remained low for 72 hours, and frequent short episodes of bradycardia occurred that were not associated with apnea and that resolved spontaneously. By 4 days of age, the heart rate was consistently above 135 bpm. The child gained weight and did well.

Samples of serum obtained from cord blood and from the infant at 12 and 38 hours had concentrations of nadolol of 43, 145, and 80 ng/ml. Breast milk obtained 38 hours post partum had a concentration of nadolol of 146 ng/ml. A T4 level obtained on day 3 was normal. The placenta weighed 240 gm and had normal histologic features.

Comment

Described is the case of an infant exposed during gestation to the β -blocker nadolol. The infant presented with cardiorespiratory depression, mild hypoglycemia, and growth retardation. The other maternal medications have not been reported to have any effects on the fetus.

The infant's depressed heart rate was probably the result of blockade by nadolol of the β-receptors of the heart. The time course and complete resolution by 72 hours support the fact that this was a pharmacologic effect. The early hypoglycemia that we observed was similar to that in infants who have prolonged in utero exposure to the β-blocker propranolol. Although the hypoglycemia might be attributed to the infant's growth retardation or mild fetal distress, β-blockade could affect the concentration of blood glucose as well. Exposure to nadolol may have contributed to the observed poor fetal growth. However, other factors cannot be easily excluded, since maternal renal disease and hypertension are known to cause intrauterine growth retardation.

We measured serum concentrations of nadolol in the infant that were within the therapeutic range for adults. Concentrations of nadolol were higher 12 and 38 hours after delivery than they were in cord blood, similar to findings with propranolol and metoprolol. The observed rise in serum concentration may reflect hemoconcentration or redistribution of tissue-sequestered drug in a non-equilibrium process.

Although the effects of exposure to propranolol on the fetus have been described, several pharmacologic properties of nadolol might put the infant at greater risk. The longer duration of action of nadolol, marketed as an advantage because of fewer daily doses required, was a disadvantage to this newborn infant. Nadolol has a half-life in adults of 17 to 24 hours. compared to the half-life of propranolol of 4 to 6 hours.2 Unlike propranolol, which is removed mainly by hepatic metabolism, nadolol is excreted unchanged by the kidneys. The infant may have experienced protracted effects of the drug because of the normally decreased glomerular filtration in the first days of life. In addition, nadolol is only 30% protein bound, unlike propranolol, which is 90% protein bound. More free. and hence active, drug would have been present in this infant and contributed to its effects. We conclude that nadolol is less desirable than propranolol for use as a β-blocker during pregnancy.

We wish to thank Walter Jump, Pharm.D., of E. B. Squibb Professional Services Division, for his help in measuring the concentrations of nadolol.

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adjacent page for brief summary.

ference: Wren BG, Brown LB, Routledge DA: Differential clinical response to oestrogens after menopause. *Med J Aust* 2:329-332, 1982.



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OGEN®

ESTROPIPATE TABLETS, USP

WARNING:

1. ESTROGENS HAVE BEEN REPORTED TO INCREASE THE RISK OF ENDOMETRIAL CARCINOMA.

Three independent case control studies have shown an increased risk of endometrial cancer in postmenopausal women exposed to exogenous estrogens for prolonged periods. 1-3 This risk was independent of the other known risk factors for endometrial cancer. These studies are further supported by the finding that incidence rates of endometrial cancer have increased sharply since 1969 in eight different areas of the United States with population-based cancer reporting systems, an increase which may be related to the rapidly expanding use of estrogens during the last decade. 4

The three case control studies reported that the risk of endometrial cancer in estrogen users was about 4.5 to 1.3.9 times greater than in nonusers. The risk appears to depend on both duration of treatment and on estrogen dose. 8 In view of these findings, when estrogens are used for the treatment of menopausal symptoms, the lowest dose that will control symptoms should be utilized and medication should be discontinued as soon as possible. When prolonged treatment is medically indicated, the patient should be reassessed on at least a semianusal basis to determine the need for continued therapy. Although the evidence must be considered preliminary, one study supposts shalt cyclic administration of low doses of estrogen may the supposts but cyclic administration of low doses of estrogen may the suppost shalt cyclic administration of low doses of estrogen may the suppost shalt cyclic administration of low doses of estrogen may the suppost shalt cyclic administration of low doses of estrogen may the suppost shalt cyclic administration of low doses of estrogen may the suppost shalt cyclic administration of low doses of estrogen may the suppost shalt cyclic administration of low doses of estrogen may the suppost shalt cyclic administration of low doses of estrogen may the suppost shalt cyclic administration of low doses of estrogen may the low of the low of the least a semiannual basis to determine the need for continued therapy. Although the evidence must be considered preliminary, one study suggests that cyclic administration of low doses of estrogen may carry less risk than continuous administration; a it therefore appears prudent to utilize such a regimen. Close clinical surveillance of all women taking estrogens is important. In all cases of undiagnosed persistent or recurring abnormal vaginal bleeding, adequate diagnostic measures should be undertaken to rule out malinanorv.

en to rule out malignancy.

There is no evidence at present that "natural" estrogens are more or less hazardous than "synthetic" estrogens at equiestrogenic doses.

2. OGEN SHOULD NOT BE USED DURING PREGNANCY.

2. OGEN SHOULD NOT BE USED DURING PREGNANCY.
According to some investigators, the use of female sex hormones, both estrogens and progestogens, during early pregnancy may seriously damage the offspring, Studies have reported that females exposed in utero to diethylstilbestrol, a non-steroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare.^{6,6} In one of these studies, this risk was estimated as not greater than 4 per 1000 exposures.⁷ Furthermore, there are reports that a high percentage of such exposed women (from 30 to 30 percent) have been found to have vaginal adenois.^{6,12} epithelial changes of the vagina and cervix. Although these reported changes are histologically benign, the investigators have not determined whether they are precursors of adeno-carcinoma.

carcinoma.

Several reports suggest an association between intrauterine exposure to female sex hormones and congenital anomalies in the off-spring, including heart defects and limb reduction defects, 13-16 One case control study. Sestimated a 4.7 fold increased risk of limb reduction defects in infants exposed in utero to sex hormones (oral contraceptives, hormone withdrawal tests for pregnancy, or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb reduction defects in exposed fetuses is somewhal less than 1 per 1000.

In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. OGEN has not been studied for these uses, and therefore should not be used during pregnancy. There is no evidence from well controlled studies that progestogens are effective for these uses.

during pregnancy. Here is no extension to the programmer are flective for these uses. If OGEN (estropipate tablets) is used during pregnancy, or if the patient becomes pregnant while taking this drug, she should be apprised of the potential risks to the fetus, and the question of continuion of the pregnancy should be addressed.

INDICATIONS AND USAGE
The cyclic administration of DGEN (estropipate tablets) is indicated for the treatment of estropen deficiency associated with (See "DOSAGE AND ADMINISTRATION" section):

- Moderate to severe vasomotor symptoms of menopause. (There is no evidence that estrogens are effective for nervous symptoms or depression which might occur during menopause, and they should not be used to treat these conditions.)
 - Atrophic vaginitis.

 - Kraurosis vulvae.
 Female hypogonadism.
 Female castration.

5. Female castation.
6. Primary ovarian failure.

OGEN (ESTROPIPATE TABLETS) HAS NOT BEEN TESTED FOR EFFICACY FOR ANY PURPOSE DURING PREGNANCY. SINCE ITS EFFECT
UPON THE FETUS IS UNKNOWN. IT CANNOT BE RECOMMENDED FOR
ANY CONDITION DURING PREGNANCY (SEE BOXED WARNING).

CONTRAINDICATIONS OGEN should get be

- CONTRANDICATIONS

 IGEN should not be used in women with any of the following conditions:

 1. Known or suspected cancer of the breast.

 2. Known or suspected estrogen-dependent neoplasia.

 3. Known or suspected manageney (See Baxed Warning).

 4. Undiagnosed abnormal genital bleeding.

 5. Active thrombophlebitis or thromboembolic disorders.

 6. A past history of thrombophlebitis, thrombosis, or thromboembolic disorers associated with previous estrogen use.

Induction of malignant neoplasms. Long-term continuous administration of

1. Induction of malignant neoplasms: Long-term continuous administration of natival and synthetic estrogers in creatian aimal species has been reported by some investigators to increase the frequency of carcinomas of the breast, cervix, vagina, and liver. There is now evidence that estrogens increase the risk of carcinoma of the endometrium in humans. [See Boxed Waming).

At the present time there is no satisfactory evidence that estrogens given to postmenopausal women increase the risk of cancer of the breast!, although a recent long-term followup of a single physician's practice has raised this possibility. Therefore, caution should be exercised when administering estrogens to women with a strong family history of breast cancer or who have breast nodules, fibrocystic disease, or abnormal mammograms. Careful breast examinations should be performed periodically.

2. Galt bladder disease. A recent study has reported a 2 to 3-fold increase in the risk of surgically confirmed gall bladder disease in women receiving post-

menopausal estrogens, 1º similar tr he 2-fold increase previously noted in users of oral contraceptives, 1º 2² In the case of oral contraceptives, the increased risk appeared after two years of use. 2²² 3. Effects similar to those caused by estrogen-progestogen oral contraceptives. There are several serious adverse effects of oral contraceptives, most of which have not, up to now, been documented as consequences of postmenopausal estrogen therapy. This may reflect the comparatively low doses of estro paisar estugent interpo, it is may return the companiery by course of eating gen used in postmenopausal women. It would be expected that the larger doses of estrogen used to treat postpartum breast engorgement would be more likely to result in these adverse effects, and, in fact, it has been shown that there is an in-creased risk of thrombosis in women receiving estrogens for postpartum breast

engorgement.^{20,21}
a. *Thromboembolic disease.* It is now well established that users of oral con traceptives have an increased risk of various thromboembolic and thrombotic traceptives have an increased risk of various thromboembolic and thrombotic vascular diseases, such as thrombophlebitis, pulmonary embolism, stroke, and myocardial infarction. 22 29 Cases of retinal thrombosis, mesenteric thrombosis, and optic neuritis have been reported in oral contraceptive users. There is evidence that the risk of several of these adverse reactions is related to the dose of the drug. 30.31 An increased risk of post-surgery thromboembolic complications has also been reported in users of oral contraceptives. 32.33 If feasible, estrogen should be discontinued at least 4 weeks before surgery of the type associated with an increased risk of thromboembolism; it should also be discontinued during periods of prolonged imposhification. periods of prolonged immobilization.

periods of prolonged immobilization.

While an increased rate of thromboembolic and thrombotic disease in postmenopausal users of estrogens has not been found that this does not rule out the
possibility that such an increase may be present or that subgroups of women
who have underlying risk factors or who are receiving relatively large doses of
estrogens may have increased risk. Therefore estrogens should not be used in
persons with active thrombophlebits or thromboembolic disorders, and they
should not be used in persons with a history of such disorders in association with
estrogen use. They should be used with caution in patients with cerebral vascular
or coronary artery disease and only for those in whom estrogens are clearly

needed.

Large doses of estrogen (5 mg conjugated estrogens per day), comparable to those used to treat cancer of the prostate and breast: have been shown in a large prospective clinical trial in men³⁻⁵ to increase the risk of nonfatal myocardial infarction, pulmonary embolism and thrombophlebitis. When estrogen doses of this size are used, any of the thromboembolic and thrombotic adverse effects associated with oral contraceptive use should be considered a clear risk.

ciated with oral contraceptive use should be considered a clear risk.

b. Hepatic adenoma. Benign hepatic adenomas appear to be associated with the use of oral contraceptives.⁵⁻⁷⁸ Although benign, and rare, these may rupture and cause death through intraabdominal hemorrhage. Such lesions have not yet been reported in association with other estrogen or progestogen preparations but should be considered in estrogen users having abdominal pain and tenderness, abdominal mass, or hypovolemic shock. Hepatocellular carcinoma has also been reported in women taking estrogen-containing oral contraceptives.⁵⁷ The relationship of this malignancy to these drugs is not known at this time.

c. Elevated blood pressure. Increased blood pressure is not uncommon in women using oral contraceptives. There is now a report that this may occur with use of estrogens in the menopause³⁹ and blood gressure should be monitored with estrogen use, especially if high doses are used.
d. Blucase tolerance. A worsening of glucase tolerance has been observed in a significant percentage of patients on estrogen-containing oral contraceptives. For this reason, diabetic patients should be carefully observed while receiving estrogen.

4. Hypercalcemia. Administration of estrogens may lead to severe hypercal cemia in patients with breast cancer and bone metastases.

PRECAUTIONS

A General Precautions.

1. A complete medical and family history should be taken prior to the initiation of any estrogen therapy. The pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen, and pelvic organs, and should include a Papanicolau smear. As a general rule, estro-gen should not be prescribed for longer than one year without another physical

gen studie for the performed.

2. Fluid retention — Estrogens may cause some degree of fluid retention. Therefore, patients with conditions such as epilepsy, migraine, and cardiac or renal dysfunction, which might be influenced by this factor, require careful obser-

vation.

3. Certain patients may develop undesirable manifestations of excessive es trogenic stimulation, such as abnormal or excessive uterine bleeding, mastodynia

etc.

4. Oral contraceptives appear to be associated with an increased incidence of mental depression.²² Although it is not clear whether this is due to the estrogenic or progestogenic component of the contraceptive, patients with a history of depression should be carefully observed.

5. Preexisting uterine leiomyemata may increase in size during estrogen use.

6. The pathologist should be advised of the patient's use of estrogen therapy when relevant specimens are submitted.

7. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence of jaundice while receiving estrogen-containing oral contraceptive therapy. If survive develops in any native freelying estrogen the

creased risk of recurrence of jaundice while receiving estrogen-containing oral contraceptive therapy. If jaundice develops in any patient receiving estrogen, the medication should be discontinued while the cause is investigated.

8. Estrogens may be poorly metabolized in patients with impaired liver function and they should be administered with caution in such patients.

9. Because estrogens influence the metabolism of calcium and phosphorus, they should be used with caution in patients with metabolic bone diseases that are associated with hypercalcemia or in patients with renal insufficiency.

8. Information for the Patient. See text on Patient Package Insert which appears after PHYSICIAN REFERENCES.

after PHYSICIAN REFERENCES.

after PHYSICIAN REFERENCES.
C. Bug Interactions. The concomitant use of any drugs which can induce hepatic microsomal enzymes with estrogens may produce estrogen levels which are lower than would be expected from the dose of estrogen administered.

The use of broad spectrum antibiotics which profoundly effect intestinal flora may influence the absorption of steroidal compounds including the estro-

Diabetics receiving insulin may have increased insulin requirements when re-

ceiving estrogens.

Laboratory Test Interference, Certain endocrine and liver function tests may

Laboratory Test Interference. Certain endocrine and liver function tests may be affected by estrogen-containing oral contraeptives. The following similar changes may be expected with larger doses of estrogen:

a. Increased porthrombia and factors VII, VIII, IX, and X; decreased antithrombin 3; increased prothrombia and factors VII, VIII, IX, and X; decreased antithrombin 3; increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by PBI, T4 by column, or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG; free T4 concentration is unaftered.

- concentration is unaltered.

 d. Abnormal glucose tolerance test results.
- Decreased pregnanediol excretion.
 Reduced response to metyrapone test.
 Reduced serum folate concentration.

g. Reduced serum folate concentration. h. Increased serum triglyceride and phospholipid concentration.
D. Carcinogenesis. Studies have shown an increased risk of endometrial cancer in postmenopausal women exposed to exogenous estrogens for prolonged periods (see Boxed Warning). At the present time there is no conclusive evidence that estrogens given to postmenopausal women increase the risk of cancer of the heast.17.40-31. There are, however, a few retrospective studies which suggest a small but statistically significant increase in the risk factor for breast cancer

among these women.^{18, 42, 44} (See "WARNINGS" section.)
E. Pregnancy. Pregnancy Category X. See "CONTRAINDICATIONS" section and Boxed Warning.
F. Nursing Mothers. Estrogens have been reported to be excreted in human breast milk. Caution should be exercised when OGEN is administered to a nursing

Wouldn't 6. Pediatric Use. Because of the effects of estrogens on epiphyseal closure they should be used judiciously in young patients in whom bone growth is not complete.

ADVERSE REACTIONS

(See Warnings regarding reports of possible induction of neoplasia, unknown ef-fects upon the fetus, increased incidence of gall bladder disease, and adverse ef-fects similar to those of oral contraceptives, including thromboembolism.) The following additional adverse reactions have been reported with estrogenic thera following additional adverse reactions have py, including oral contraceptives:

1. Genitourinary system.
Increase in size of uterine fibromyomata.
Vaginal candidiasis.
Cystitis-like syndrome.
Dysmenorrhea.
Amenorrhea during and after treatment.
Chance in ceptical eversion and in denre

Change in cervical eversion and in degree of cervical secretion.

Breakthrough bleeding, spotting, change in menstrual flow Premenstrual-like syndrome.

Breast

Z. Breast.
Tenderness, enlargement, secretion.
3. Gastrointestinal.
Cholestatic jaundice.

Vomiting, nausea. Abdominal cramps, bloating.

4 Skin

Hemorrhagic eruption. Erythema nodosum. Erythema multiforme.

Hirsutism. Chloasma or melasma which may persist when drug is discontinued. Loss of scalp hair.

Eves.

Steepening of corneal curvature Intolerance to contact lenses

CNS

6. CNS.
Chorea.
Mental depression.
Migraine, dizziness, headache
7. Miscellaneous.
Aggravation of porphyria.

Reduced carbohydrate tolerance. Increase or decrease in weight

Changes in libido.

OVERDOSAGE

Numerous reports of ingestion of large doses of estrogen-containing oral contra-ceptives by young children indicate that serious ill effects do not occur. Overdos-age of estrogen may cause nausea and withdrawal bleeding may occur in

DOSAGE AND ADMINISTRATION

Given cyclically for short-term use:
 For treatment of moderate to severe vasomator symptoms, atrophic vaginitis, or kraurosis vulvae associated with the menopause.

or Krauross vurvee associated with the menopause.

The lowest dose that will control symptoms should be chosen and medication should be discontinued as promptly as possible.

Administration should be cyclic (e.g., 3 weeks on and 1 week off).

Attempts to discontinue or taper medication should be made at 3 to 6 month.

OGEN 5 Tablet per day. The lowest dose that will control symptoms should be chosen. If the patient has not menstruated within the last two months or more

cyclic administration is started arbitrarily. If the patient is menstruating, cyclic administration is started on day 5 of bleeding.

Atrophic vaginitis and kraurosis vulvae — One OGEN .625 Tablet to one OGEN 5 Tablet daily, depending upon the tissue response of the individual patient. The lowest dose that will control symptoms should be chosen. Administer cyclically.

2. Given cyclically:

Female hypogonadism; female castration; primary ovarian failure.

Emale hypogonadism; female castration; primary ovarian failure.

Usual dosage ranges:

Female hypogonadism — A daily dose of one OGEN 1.25 Tablet to three
OGEN 2.5 Tablets may be given for the first three weeks of a theoretical cycle,
followed by a rest period of eight to ten days. The lowest dose that will control
symptoms should be chosen. If bleeding does not occur by the end of this period,
the same dosage schedule, is repeated. The number of courses of estrogen therapy necessary to produce bleeding may vary depending on the responsiveness of
the endometrium. If satisfactory withdrawal bleeding does not occur, an oral progestogen may be given in addition to estrogen during the third week of the cycle.

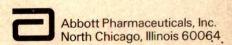
Female castration and primary ovarian failure — A daily dose of one
OGEN 1.25 Tablet to three OGEN 2.5 Tablets may be given for the first three
weeks of a theoretical cycle, followed by a rest period of eight to ten days
diguist dosage upward or downward according to severity of symptoms and response of the patient. For maintenance, adjust dosage to lowest level that will
provide effective control.

Treated patients with an intact uterus should be monitored closely for signs of
endometrial cancer and appropriate diagnostic measures should be taken to rule
out malignancy in the event of persistent or recurring abnormal vaginal bleeding.

HOW SUPPLIED

HOW SUPPLIED OBEN (estropinghet tablets, USP) is supplied as 06EN. 825 (0.75 mg estropinate), yellow tablets, NDC 0074-3943-04. 06EN 1.25 (1.5 mg estropinate), peach-colored tablets, NDC 0074-3946-04; 06EN 2.5 (3 mg estropinate), lught green tablets, NDC 0074-3951-04; and 06EN 5 (6 mg estropinate), light green tablets, NDC 0074-3951-104; and 06EN 5 (6 mg estropinate), light green tablets, NDC 0074-3951-13. Tablets of all flour dosage levels are standardized dosage flexibility. All tablet sizes of 06EN are available in bottles of 100.

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Current Development

The nonstress test as a diagnostic test: A critical reappraisal

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In the past decade the nonstress test has become a major method of assessing high-risk pregnancy. Although many studies have been published, there has been a lack of rigorous adherence to the standard criteria for diagnostic testing, that is, presentation of test specificity, sensitivity, predictive value, and the prevalence of abnormal outcomes in the populations studied. Furthermore, the populations studied vary widely in composition, testing conditions, methods of test interpretation, and clinical management. The authors undertake a review of these studies, with a focus on these issues, in an attempt to indicate potential problems involved in current test usage and to suggest avenues for needed clinical investigation. (AM J OBSTET GYNECOL 1985;152:1047-53.)

Key words: Nonstress test, sensitivity, specificity, predictive value

Electronic recording of fetal heart rate has been an accepted diagnostic procedure for nearly two decades. Early studies1.2 of fetal heart rate patterns were based on recordings from either intrapartum patients or antepartum patients who delivered within hours of their last tracing. These investigations had the obvious advantage of immediate correlation between specific fetal heart rate patterns and actual perinatal outcome. Antepartum testing schemes, based on these data, evolved as contraction stress tests or nonstress tests and have been used to evaluate fetal risk for intrauterine compromise. Clinical use of these tests has been associated with decreased rates of perinatal mortality and morbidity.3.4 Since the mid-1970s when the nonstress test was introduced in the United States,5,6 more than 100 articles supporting its usefulness have appeared in the English language literature. Because of its ease of administration and lack of contraindications, the nonstress test has become a standard method for antepartum diagnosis and management in many American obstetric units.

Although the nonstress test has been considered to be an objective assessment of fetal condition, there has been little effort to determine how well it satisfies the basic requirements of a laboratory test. These requirements include the determination of sensitivity, specificity, predictive value, and the prevalence of abnormal

outcomes in the population studied. The current status of the nonstress test has also been confused by the vast differences in the obstetric populations tested and the great variety of interpretative criteria and clinical responses to "abnormal" test results. Thus clinicians who perform nonstress tests must not only select among the different schemes available and adhere strictly to the prescribed testing conditions, but they must know how closely their population resembles the one for which the test criteria and management protocols were originally reported.

Previous reviews of the nonstress test⁸⁻¹⁰ have raised some of the problems inherent in antepartum testing but none has critically examined the issues mentioned above. Our analysis of major studies from the past decade therefore places special emphasis on the composition of the study populations' testing conditions, interpretative criteria, clinical response to test results, and compliance with the essential requirements for a laboratory test. Our ultimate goal was to assess the current diagnostic value of the nonstress test and to indicate areas that require further investigation.

Material and methods

A survey of English language publications from 1974 to 1984 was performed by use of the National Library of Medicine MEDLINE search. All articles containing in the key words, title, or abstract any references to nonstress test, unstressed or resting fetal heart rate testing, fetal heart rate acceleration determination, or antepartum cardiotocography were reviewed by us. Articles were included in our analysis only if they met the

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Table I. Matrix of diagnostic test results and perinatal outcomes

	Abwrmal perinatal ouwome*	Normal perinatal outcome	Total
Abnormal test result (nonreactive)	a	C	a + c
Normal test result (reactive)	ь	d	b + d
Total	a – b	c + d	a + b + c + d

Sensitivity = $\frac{a}{a+b}$. Negative predictive value = $\frac{d}{b+d}$. Prevalence = $\frac{a+b}{a+b+c+d}$. Specificity = $\frac{d}{c+d}$. Positive predictive value = $\frac{a}{a+c}$.

*Defined as perinatal mortality, intrapartum fetal distress, neonatal depression, other neonatal morbidity, and intrauterine growth retardation.

following criteria: (1) they were population studies, not case reports; (2) population size was greater than 100: (3) they were original studies, not reviews; (4) nonstress test results were correlated with perinatal outcomes: (5) nonstress tests were used in clinical management.

Definitions (Table I). Sensitivity referred to the proportion of patients with abnormal perinatal outcomes who had abnormal tests. Specificity was the proportion of patients with normal perinatal outcomes and normal tests. Positive predictive value was the proportion of patients with an abnormal test who had adverse perinatal outcomes. Negative predictive value was the proportion of patients with a normal test who had normal perinatal outcomes. Prevalence was the incidence of abnormal perinatal outcomes, defined as mortality, intrapartum fetal distress, neonatal depression or complications, and intrauterine growth retardation, occurring in the study group as a whole.

Each study group was examined for major high-risk conditions and gestational age range. When available, details of testing conditions, interpretative criteria, and response to test results were also analyzed. When it was appropriate, multiple comparisons were performed with use of χ^2 tests.

Results

Forty-five articles^{5, 6, 11-53} met the inclusion criteria of this study. The journals represented were AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY (20 articles), Obstetrics and Gynecology (15), Journal of Reproductive Medicine (3), British Journal of Obstetrics and Gynaecology (5), British Medical Journal (1), and Seminars in Perinatology (1). Overall this survey included 24,407 fetuses undergoing 49,403 tests.

Sensitivity, specificity, predictive value, and prevalence. Only six studies actually presented these parameters in their texts or tables. These values could be calculated from the data available in an additional 27 reports; however, in 12 studies, or 26.7% of the total,

this information was not presented or determinable after a careful review of the data. For the 33 studies with adequate information the mean sensitivity was 48% (range: 11% to 100%); mean specificity, 93% (range: 56% to 100%); mean positive predictive value, 38% (range: 1% to 86%); mean negative predictive value, 95% (range: 72% to 100%) (Figs. 1 and 2). The mean prevalence of abnormal outcomes for these studies was 12% (range: 0% to 38%).

Composition of study populations. Each study group was analyzed for size, gestational age range, and incidence of high-risk conditions. Populations ranged in size from 101 to 2003 (mean, 541) and in gestational age from 24 to 47 weeks. The most common test indications in order of frequency were postdatism, hypertensive disorders, and intrauterine growth retardation. Table II shows that the prevalence of high-risk conditions differed considerably among the reported studies. Only six studies^{27, 30, 35, 43, 45, 46} were similar enough to be considered as subsets of the same population (χ^2 , p > 0.05). In 20 studies the gestational age of the fetuses tested was not available, and in eight studies the incidence of high-risk conditions was not tabulated.

Testing conditions, test frequency, and interval to delivery. Although 32 authors described their testing methodology, only two^{37, 48} provided details of their observation methods, data validation, and maternal state variables. Twelve articles did not specify testing frequency, but 33 indicated that the nonstress test was performed once or twice per week. The actual intervals between the last nonstress and delivery were unavailable in 18 instances, and when specified, there was a range of 60% to 100% for patients who were delivered within 7 days of their last nonstress test. In only 17 studies were all fetuses delivered within 7 days of their final test.

Criteria for test interpretation. Only one report³⁴ failed to describe objective criteria for nonstress test

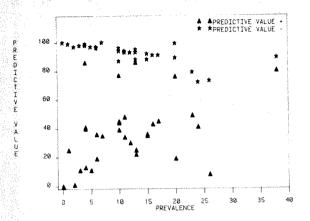


Fig. 1. Disease prevalence versus predictive value.

reactivity. The remaining 44 studies used 21 different standards for a "reactive" nonstress test (Table III). Although the most common criterion for reactivity was the presence of at least 2 fetal heart rate accelerations of at least 15 bpm amplitude and 15 seconds' duration in any 20-minute epoch, most (26 of 45) used other interpretative criteria. The correlation between nonstress test interpretative criteria and diagnostic parameters (sensitivity, specificity, etc.) is also listed in Table III. Although the diagnostic values were improved by use of a minimum threshold of 5 accelerations in a 20minute window, it should be noted that there were several other approaches that yielded excellent sensitivity and positive predictive values with minimal compromise of specificity or negative predictive values. The first (Rochard et al.6) was unique having been based on a population with a very high percentage of Rh-sensitized pregnancies and having included baseline variability in its interpretative scheme. In this report, sensitivity and positive predictive value both exceeded 80%. Brown and Patrick 37 improved their positive predictive value (86%), negative predictive value (98%), and specificity (99.7%) by extending their observations for as long as 120 minutes. Finally, Krebs and Petres¹⁰ achieved relatively high sensitivity (60%), specificity (99%), and positive predictive value (86%) by the use of a multiple parameter scoring system that included the evaluation of baseline fetal heart rate, fetal heart rate variability, and fetal movements.

Clinical response to nonstress test outcome. Twelve articles did not clearly indicate their strategies for subsequent management of an abnormal or "nonreactive" nonstress test. When these data were available, primary management techniques were evenly divided between test repetition within 24 hours (11 studies) and fetal stimulation by physical manipulation, sound signals, or maternal administration of glucose or orange juice (15 studies). Twenty-six studies performed contraction stress tests as a backup for nonstress tests that remained nonreactive or failed to respond to fetal stimulation.

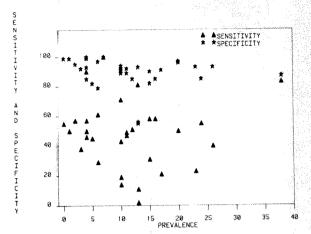


Fig. 2. Disease prevalence versus sensitivity and specificity.

Only seven groups proceeded directly from a nonreactive nonstress test, without a contraction stress test, to delivery. There was no difference in the prevalence of abnormal outcomes when the contraction stress test was used as a backup test (11%) or when no contraction stress test was performed (12%).

Comment

Although all of the reviewed studies purported to demonstrate the diagnostic value of nonstress testing, most (80%) failed to provide adequate qualitative or quantitative data for all aspects of our critical evaluation. The gestational age range of the fetuses undergoing testing was the most frequently omitted information. This is a major deficiency, since gestational age influences the incidence of "reactivity" in the early third trimester^{39, 54, 55} as well as the nature of a "reactive" fetal heart rate baseline response.³⁹

Sixteen percent of the studies did not indicate the incidence of high-risk conditions leading to fetal heart rate testing. The incidence of medical/obstetric problems influences patient selection for testing and therefore may affect the prevalence of adverse perinatal outcomes. Only six groups could be considered subsets of the same population; however, they were assessed by four different interpretative criteria as well as different testing protocols, which made it impossible to determine valid cumulative predictive values, sensitivity, or specificity.

The reviewed studies did not share uniform test conditions of maternal state, length of observation, or frequency of testing; only two studies^{37, 48} presented all of this information in detail. The potential influences of maternal fasting, smoking, and physical activity on fetal heart rate baseline have been well described⁵⁶⁻⁵⁸ as has been the relationship of observation period length to the incidence of fetal heart rate accelerations.^{37, 35} The failure to account for all of these favors raises concerns

Table II. Percentage distribution of high-risk conditions and diagnostic values in study populations

Study	Post- dates	Hyper- tension	Intrauterine growth retardation	Diabetes mellitus	Rh-iso- immunization	Other	Sensitivity	Specificity	Positive predictive value	Negative predictive value	Prevalence
Rochard et al.6	7	20	0	10	50	13	83	37	80	89	38
Schifrin et al. 12	10	10	0	5	0	75	ND	ND	ND	ND	ND
Lee et al.5	54	23	. 0	23	0	0	50	99	25	99	11
Lee and Drukker ¹¹	40	27	2	10	0	21	50	97	19	99	12
Keegan and Paul ²⁴	58	6	15	11	2	8	ND	ND	ND	ND	ND
Barrett et al.35	31	- 16	14	11	1	27	ND	NĎ	ND	ND	ND
Phelan ³⁶	42	15	25	10	0.4	7.6	45	82	11	97	5
Bishop ³⁸	14	27	8	9	0	42	ND	ND	ND	ND	ND
Devoe ²⁷	21	18	26	9	0	26	51	85	30	93	12
Flynn and Kelly ¹⁹	38	10	33	1	3	15	71	91	39	96	10
Flynn et al.20	0	0	19	0	0	81	ND	ND	ND	ND	ND
Brown and Patrick ³⁷	4	26	11	11	ő	48	50	99	.86	98	4
Beischer et al. ⁵¹	9	26	24	5	0	36	23	93	49	79	23
Aladjem et al. ³⁹	10	24	6	21	0	39	19	89	45	87	10
Krebs and Petres ⁴⁰	19	45	8	15	1	12	60	99	86	93	13
Nochimson et al. ¹⁴	63	19	4	. 2	0.5	11.5	1.1	55	1	97	2
Koller and Curet ¹⁵	18	32	19	31	0	0	ND	ND	ND	ND	ND
Rayburn et al. ¹⁶	34	25	3	18	.4	16	57	95	40	98	4
Merkur ¹⁷	17	26	8	2	0	47	46	99	77	96	10
Pratt et al. ²³	17	32	13	25	1	12	14	92	41	72	24
Mendenhall et al. ²⁵	4	28	12	10	1	46	55	85	19	97	6
Devoe et al.43	26	24	23	10	1	16	61	79	45	91	17
Lyons et al.42	5	. 28	9	13	8	37	ND	ND	ND	ND	ND
Baskett et al.47	18	15	15	4	0	48	21	91	25	88	13
Goldkrand and Benjamin ⁴⁵	42	22	11	12	0	15	2	93	8	73	26
Manning et al. ⁵⁰	22	27	0	15	ł	35	57	85	13	98	4
Lofgren ³⁴	5	15	15	0	0	65	ND	ND	ND	ND	ND
Rayburn et al. ²⁸	36	26	2	21	4	11	29	97	36	96	6
O'Leary et al.30	38	14	13	12	0	23	47	89	34	94	- 11
Lauersen et al. ³³	31	8	. 0	22	Ó	39	ND	ND	ND	ND	ND
Gibbons and Nagle ³¹	28	14	20	34	θ	4	90	93	41	99	4
et al.44	38	7	. 1	22	0	32	31	90	36	88	15
Platt et al.46	37	13	10	22	0	18	43	94	44	94	10
	17	23	19	14	0.6	26.4	100	100	35	100	ž
	43	7	11	6 -	2	31	58	85	43	91	16
	34	12	8	31	4	11	58	82	35	92	15 15
	40	15	20	7	1	17	49	92	48	93	11
Range	0-63 25.7	0-32 18.9	0-33 12.4	1-34 13.1	0-50 2.3	0-81 24.9					

ND = Not determinable.

about the reproducibility of test data in any study and the validity of comparing the results from different studies.

Test interpretation and clinical response to abnormal

tests are inextricably linked and must be considered together. We identified 21 different interpretative criteria, most of which were based on the presence or absence of accelerations in a limited time period. None

Table III Interpretative criteria for NST reactivity*

nu	nimum mber of lerations	Minimum acceleration amplitude (bpm) + duration (sec)	Minimum diagnostic window (min)	Number of studies†	Mean sensitivity (%)	Mean specificity (%)	Mean positive predictive value (%)	Mean negative predictive value (%)
	1	NS + NS	20	l 16	57	95	40	98
	i	10 + NS	30	125	55	85	19	96
	i	10 + NS	60	129	ND	ND	ND	ND
	2	10 + 15	20	111	ND	ND	ND	ND
	2	15 + 15	10	312,35,49	58	82	35	95
	9	15 + 15	20	824,28,45-47,50,52,53	37	91	34	90
	9	15 + 30	30	125	14	92	41	72
	9	15 + 30	20.	153	58	82	35	92
	3	10 + 15	15	l 15	ND	ND	ND	ND
	3	15 + 15	20	233,44	31	.90	36	88
	3	15 + 15	30	227,38	51	85	30	93
	4	15 + 15	20	55.14,19.30.36	45	83	22	97
	5	15 + 15	20	618,26,31,32,37,48	65	95	44	98

NS = Not specified, ND = not determinable.

of the numerical criteria for reactivity, however, bore a mathematical relationship to the nomographic data of Patrick et al. 59 or Visser et al. 60 These investigators reported that normal fetuses exhibited mean acceleration frequencies, ranging from 19.6 to 34 per hour, depending on whether a minimum amplitude threshold of 15 or 10 bpm was chosen. Of the different maneuvers used to "awaken" nonreactive fetuses, both manual stimulation and glucose administration have been shown, subsequently, not to produce significant alterations in fetal heart rate reactivity.^{58, 61, 62}

These studies presented a wide range of test-to-delivery intervals. In only one report4 were all fetuses delivered within 24 hours of their last nonstress test; however, this study lacked sufficient data to determine test sensitivity, specificity, or predictive values. The relationship of the predictive value of the nonstress test to the length of the test-to-delivery interval is an important issue, which has not been adequately addressed in the studies that we examined. Anecdotal reports of false negative nonstress tests and the occasional occurrence of unpredictable obstetric accidents suggest that this issue may be difficult to resolve.

A valid laboratory test should provide established sensitivity, specificity, and predictive values. Unfortunately, 13 of the 45 studies examined could not meet these criteria for a variety of reasons: (1) the data were not clearly presented; (2) all perinatal outcomes were not available; (3) all test results were not available; (4) nonstandard or incomplete endpoints were chosen. More importantly, very few (6 of 45) articles explicitly reported these diagnostic parameters. Casscells et al.63 have noted that many clinicians are ill-equipped to apply such data, even when available. Therefore, authors must present these data and discuss their implications as explicitly as possible.

Diagnostic test parameters are interdependent,7 i.e., changing the threshold for abnormality has reciprocal effects on sensitivity and specificity whereas altering the prevalence of adverse outcomes will have reciprocal effects on negative and positive predictive values. Consequently, as suggested by Ransohoff and Feinstein,64 the prevalence of abnormal clinical outcomes in any study population must be known or at least closely estimated if appropriate diagnostic thresholds are to be set and realistic senstivity figures and positive predictive values are to be obtained. For individual obstetricians managing smaller, less heterogenous populations than those cited in the studies, this process may prove to be extremely difficult to perform. Hence a diagnostic approach that uses the individual fetus as its own control may be a more valid and practical alternative; this should be explored by future studies.

We have analyzed a large number of studies in order to assess the current status of the nonstress test as a diagnostic test. Although it is impossible to reconcile the totality of these current studies, we believe that some useful observations can be made. First, there are minimum diagnostic values that should be satisfied by any future study: (1) sensitivity, 50%; (2) specificity, 94%; (3) positive predictive value, 50%; (4) negative predictive value, 94%. These figures are derived from an application of Bayes' theorem, with assumption of a disease prevalence of 10%. Those studies that achieved these standards differed in their interpretative criteria and indications for testing. Their common feature was the additional use of other fetal heart rate data, either baseline rate or baseline variability, and the

^{*}Eight additional interpretative schemes were reported: (1) accelerations (nonstress) and baseline variability >6 bpm, ^{6,51} (2) two different fetal movements: acceleration ratios, ^{22,39} (3) beat-to-beat variability alone >10 bpm, ^{13,17,20} and (4) four different multiple-parameter scoring systems. ⁴⁰⁻⁴³

[†]One study did not report its reactivity criteria.

requirement for acceleration frequency >2 per 20 minutes. Extending initially nonreactive tests for more than 40 minutes may also improve diagnostic accuracy, but physical or biochemical stimulation is probably useless. Irrespective of interpretative criteria, test sensitivity was greatest when the population contained high percentages of Rh-sensitized or diabetic pregnancies. The lowest test sensitivity and positive predictive values were found in populations with high percentages of postdate pregnancies. Additional studies are needed, with use of uniform testing conditions, to define the relationship between optimal interpretative criteria for specific pregnancy complications under surveillance. It may then become possible to ascertain whether the nonstress test should be the primary diagnostic test for each of these problems.

If the nonstress test is to remain an important diagnostic modality, the issues of interpretative criteria, test conditions, and population composition must be reconsidered. In the future, authors must specify these data in detail and clearly present their parameters of sensitivity, specificity, predictive values, and prevalence. They would also be well advised to consider the value of adding other fetal heart rate information such as baseline rate and variability to their interpretative criteria. Because clinical management, including obstetrical intervention or nonintervention, may be influenced or directly follow the outcome of a nonstress test, it is even more important that the questions we have raised be conscientiously pursued by clinical investigators.

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The effect of training in microsurgery

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One of the most important and fundamental prerequisites for successful microsurgery is serious and adequate training. This article presents the effect of microsurgical training on the functional end result of reanastomosis in the rabbit fallopian tube and evaluates the appropriate duration of laboratory training. In 50 New Zealand White female rabbits a microsurgical reanastomosis of the fallopian tube in its isthmic portion was performed. The rabbits were divided into five groups. In the first group only 30% of the rabbits conceived on the side operated on. The nidation index was only 0.269 as compared to 0.831 for the control side. Improvement in microsurgical skill following 100 isthmic anastomoses resulted in a 100% pregnancy rate for the last group. The nidation indexes in this group were the same on the side operated on and on the control side, 0.774 and 0.702, respectively. Scanning electron microscopic examination of the anastomosis site revealed a progressive increase in pattency rate and in the amount of apposed mucosal folds in the three groups. A training program is recommended, which should bring about a nidation index and pregnancy rate that should not differ from the side operated on to the control side. (AM J OBSTET GYNECOL 1985;152:1054-8.)

Key words: Microsurgery, effect of training

In gynecologic infertility surgery the results achieved with the conventional macrosurgical techniques have not been satisfactory. The conception rate has been disappointingly small. Siegler and Hellman' reported a pregnancy rate less than 8% in a group of 191 patients who underwent tubal midportion reanastomosis by macrosurgical techniques. Others^{2, 3} also reported low pregnancy rates following the use of similar techniques. There are, however, some other articles reporting higher pregnancy rates without use of the operating microscope. Peterson et al.⁴ reported a 50% term pregnancy rate following uterotubal implantation after previous sterilization. Recently, Siegler and Kontopoulos⁵ reported an overall term pregnancy rate of 12.5% in 80 patients undergoing macrosurgical tubal repair.

Stimulated by the results achieved with microsurgical methods in other surgical disciplines, 6.7 gynecologists have begun to incorporate the operating microscope in their specialty for reversal of sterilization and other tubal and ovarian reconstructive operations. Those

techniques have particularly improved the results of reanastomosis in tubal surgery. Winston⁸ reported a 65% intrauterine pregnancy rate after microsurgical tubocornual anastomosis for reversal of sterilization and Gomel⁹ 72.7%. Eddy et al.¹⁰ reported a 100% pregnancy rate in rabbits following microsurgical oviduct repair.

One of the most fundamental prerequisites and a sine qua non for successful microsurgery is adequate training in order to produce the dexterity, coordination, and ability. Staged exercise provides the fundamental background necessary prior to the application of these techniques in clinical microsurgery. The purpose of the present investigation is twofold: First, to report on the effect of microsurgical training on the results of rabbit oviduct reanastomosis, and second, to evaluate the appropriate duration of laboratory training.

Material and methods

Female New Zealand White rabbits weighing between 2500 and 3500 gm were operated on. General anesthesia was induced with Hypnorme* (0.4 ml/kg of body weight) and continued with a gas mixture of nitrous oxide, oxygen, and fluothane administered by mask. The animal was secured to the operating table, and the

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Table I. The effect of training on pregnancy rate and nidation index (mean \pm SE)

	D	Nidation	n index
Group	Pregnancy rate (%)	Operation side	Control side
Group 1 (anastomoses 1-10)	30	0.269 ± 0.089	0.831 ± 0.277
Group 3 (anastomoses 51-60)	70*	0.574 ± 0.191	0.878 ± 0.292
Group 5 (anastomoses 101-110)	100	0.774 ± 0.258	0.702 ± 0.234

^{*}p < 0.05 versus group 1.

Table II. The effect of training on patency rate and mucosal folds apposition at anastomosis site (Mean \pm SE)

	Group	Patency rate	Percentage of apposed mucosal folds	
	Group 1 (anastomoses 1-10)	37.2 ± 6.6*†	18.3 ± 8.5*†	
	Group 3 (anastomoses 51-60)	$57.5 \pm 9.5 \ddagger$	$28.0 \pm 10.2 \ddagger$	
19 13 14	Group 5 (anastomoses 101-110)	81.8 ± 5.4	71.7 ± 8.2	

^{*}p < 0.005 versus group 3.

flanks were shaved. Before operation the animals were fasted for 16 hours.

Microsurgical techniques. The Carl Zeiss operating microscope No. 6 equipped with a remote foot panel control for focus and magnification was used. Microsurgical principles, including continuous irrigation, gentle tissue handling, and meticulous hemostasis with bipolar coagulation, were practiced during the operation. The isthmus, with a part of the uterus, was exteriorized through a flank incision and put on a minioperating table covered with a silicone rubber sheet. The superior mesotubarium was grasped and cut. Tiny blood vessels were carefully coagulated. The blood vessels in the mesotubarium were cleared from fat tissue, coagulated with bipolar diathermy, and sectioned. Two Winston tubal clamps11 were applied across the isthmus preventing bleeding from the cut ends. These clamps compress the vessels in the tubal wall and the vessels immediately adjacent to the tube without damaging the delicate epithelium of the oviduct. The isthmus between the clamps was cut with Castro-Viejo-Vannas microscissors. End-to-end anastomosis was performed with 11-0 nylon sutures mounted on a 3/8 circular tapered needle. After the tube was splinted with a polyethylene splint 0.4 mm in diameter, six to seven interrupted sutures were placed around the perimeter of the isthmus through the serosa and myosalpinx, excluding the endosalpinx. The mesothelium of the isthmus and the superior mesotubarium were repaired with several interrupted sutures. The uterus and the tube were carefully repositioned and the flank incision closed.

One hundred ten anastomoses were performed by a surgeon who did not have any microsurgical experience. The results of the anastomoses, Nos. 1 to 10 (group 1), 51 to 60 (group 3), and 101 to 110 (group 5) were evaluated in order to demonstrate the effect of training. In these animals one isthmic anastomosis was performed, the contralateral oviduct serving as a control side. Four isthmic anastomoses, two on each oviduct, were performed in the other rabbits (anastomoses Nos. 11 to 50 in group 2 and 61 to 100 in group 4) in order to gain microsurgical experience. These anastomoses (groups 2 and 4) will not be discussed further.

Three weeks after the microsurgical reanastomosis, the rabbits in the three study groups (1, 3, and 5) were mated with a buck of established fertility. Ten to 14 days later laparoscopy was performed and the number of corpora lutea and implantation sites were counted on each side and the nidation index calculated.

The effect of training was evaluated by the following parameters: (1) the pregnancy rate, which is the percentage of rabbits who conceived following the operation on the operated side; (2) the nidation index, which is defined as the number of blastocysts divided by the number of corpora lutea on each side; (3) the anatomic evaluation of the anastomosis site. For evaluation, the oviducts with the anastomosis were resected. The specimens approximately 1 cm long were opened longitudinally with Castro-Viejo-Vannas microscissors under the operating microscope. The specimens were stained for seanning electron microscopic evaluation. The degree of stenosis and the percentage of apposed mucosal folds (continuity of four to five main folds) were mea-

tp > 0.05 versus control side.

 $[\]dagger p < 0.005$ versus group 5.

p < 0.005 versus group 5.

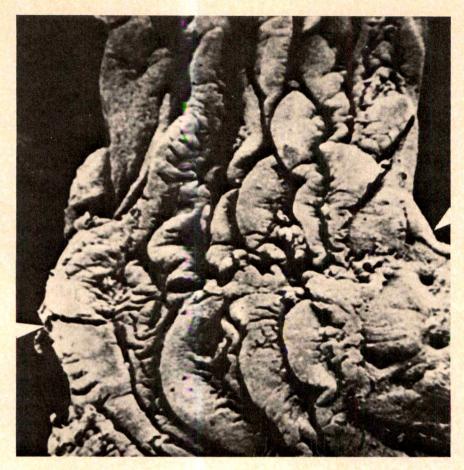


Fig. 1. Isthmic endosalpinx following microsurgical anastomosis. There is no stenosis and the mucosal folds are well apposed at the anastomosis site (arrows).

sured at the anastomosis site on pictures taken via the electron microscope.

All morphologic observations were performed in the absence of knowledge of the group to which the animals belonged. Significance was evaluated by Student's paired and unpaired *t* test.

Results

Both pregnancy rates and nidation indexes increased significantly by training. The first 10 anastomoses resulted in a pregnancy rate of only 30% and a nidation index of 0.269. The fiftieth to sixtieth anastomoses resulted in a pregnancy rate of 70% and a nidation index of 0.574, and after 100 anastomoses the pregnancy rate and nidation index, respectively, were 100% and 0.774 (Table I). The following histologic results were obtained on evaluation of the anastomosis site: 37.2% patency of the oviduct lumen in group 1, 57.5% in group 3, and 81.8% in group 5. With regard to the number of mucosal folds apposed, there were 18.3% in group 1, 28% in group 3, and 71.7% in group 5 (Table II) (Figs. 1 and 2).

Comment

The improvement of microsurgical results is clearly demonstrated to be a direct effect of training. In the first group only three rabbits conceived on the side operated on. The nidation index, which expresses the efficacy of the tube on each side, was only 0.269 as compared to 0.831 for the control side. Improvement in microsurgical skill following 100 isthmic anastomoses resulted in a 100% pregnancy rate for the last 10 rabbits. The nidation index in this group was the same on the side operated on and on the control side, 0.774 and 0.772, respectively.

Scanning electron microscopic examination of the anastomosis site revealed a direct correlation with the pregnancy rates and the nidation index. Seven of the oviducts in group 1, three in group 2, and none in group 5 had stenosis of more than 50%.

Improvement was noted in the apposition of the mucosal folds with 18% apposition in group 1, 28% in group 3, and 71% in group 5. The differences between groups 1 and 5, between groups 1 and 3, and between groups 3 and 5 are statistically significant (Table II).



Fig. 2. Isthmic endosalpinx following microsurgical anastomosis. Note the severe stenosis at the anastomosis site (arrows).

The progressive increase in patency in the three groups of animals and the increase of apposed mucosal folds at the anastomosis site coincide with the increased pregnancy rate and nidation index obtained. In order to achieve a 100% pregnancy rate and a nidation index of 0.774, the tube at the anastomosis site should be 81% patent and at least 72% of the folds should be apposed.

It could be argued that an inexperienced microsurgeon can perform tubal anastomosis, provided that he has the necessary equipment and that he knows the technique. This is, to our knowledge, the first demonstration that the end result of tubal anastomosis is improved by microsurgical training. Indeed, both nidation index and pregnancy rate increased progressively. This was explained by improvement of the degree of patency and the percentage of apposed mucosal folds. Therefore, training not only improves the skill but definitely improves the functional end result of an anastomosis.

In microsurgery, therefore, skill should be gained in the laboratory before one attempts to operate on the human. The acquired skill can then be applied with safety to patients in the operating room without unnecessary frustrations, failures, and possible harm. It is difficult to say how many tubal microsurgical operations a gynecologic surgeon should perform before using his skill on the human. The actual number of anastomoses in a training program may vary from surgeon to surgeon. We would, therefore, recommend a training program that should bring about a nidation index and pregnancy rate that should not differ from the side operated on to the control side. Only at that

point are his knowledge and skill good enough to be applied to humans.

Appreciation is expressed to Lieve Desmect and Andre Bergen for their continuous technical assistance.

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Effect of isoproterenol on uterine blood flow and cardiac output distribution in pregnant guinea pigs

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The effects of isoproterenol, 0.05 μg·min⁻¹·kg⁻¹ infused intravenously for 2 hours, on cardiac output distribution and uteroplacental blood flow were studied in six chronically catheterized guinea pigs between 60 and 68 days of pregnancy. Isoproterenol caused marked cardiac stimulation: Cardiac output was increased by 41%, of which 70% was distributed to the carcass and gastrointestinal tract. Absolute placental blood flow remained essentially unchanged, but the placental fraction of cardiac output decreased from 16% to 11%. Myometrial blood flow increased by 72%. Uteroplacental vascular resistance did not change significantly. In the guinea pig in late pregnancy β-adrenergic receptors are present in the myoendometrial vessels but could not be demonstrated in the maternal vessels supplying the placenta. (AM J OBSTET GYNECOL 1985;152:1058-62.)

Key words: Uterine blood flow, cardiac output distribution, isoproterenol, guinea pigs, microspheres

Although β-adrenergic agonists are widely used in clinical obstetrics for the pharmacologic inhibition of premature labor, the effects of the classical β-adrenergic agonist isoproterenol on the circulation during pregnancy have been studied only in sheep. ¹⁻⁶ The sheep as a model in the study of human uteroplacental blood flow, especially vasodilatation, has been criticized

by Bell,⁷ who instead proposed the guinea pig. We therefore decided to investigate the effects of isoproterenol on the circulation of pregnant guinea pigs, using a chronic preparation to avoid the stress of operation and microspheres to measure changes in the distribution of cardiac output as well as regional blood flow distribution within the uterus.

Material and methods

Six primigravid albino guinea pigs with known pregnancy durations⁸ were used in this study. Between the days 47 and 51 of pregnancy polyethylene catheters (inside diameter, 0.58 mm; outside diameter, 0.96 mm) were introduced into the left ventricle via the left carotid artery, right external jugular vein, and abdominal

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Table I. Cardiovascular measurements before, during, and after infusion of isoproterenol in pregnant guinea pigs

Parameter	Control	Isoproterenol	Recovery
Maternal heart rate (bpm)	244 ± 3	286 ± 8*	231 ± 14*
Mean arterial pressure (mm Hg)	58 ± 3	57 ± 3	55 ± 2
Cardiac output (ml·min ⁻¹)	281 ± 32	397 ± 47*	297 ± 52*
Cardiac output (ml·min ⁻¹ · 100 g ⁻¹)	24.5 ± 1.8	$34.6 \pm 2.7*$	25.6 ± 3.5*
Stroke volume	1.15 ± 0.12	$1.40 \pm 0.18*$	1.27 ± 0.19
Total periph- eral resis- tance	2.47 ± 0.28	$1.72 \pm 0.21*$	2.34 ± 0.28*
Uteroplacental vascular resistance	0.20 ± 0.04	0.20 ± 0.03	0.17 ± 0.03

Values are means ± SEM.

aorta (below the level of the renal arteries) via the left femoral artery with the animal under general anesthesia with ketamine hydrochloride (45 mg · kg⁻¹ subcutaneously) and xylazine (4 mg \cdot kg⁻¹ intramuscularly). The areas of the skin incisions were also infiltrated with Xylocaine (1%). Hypothermia during the operation and recovery period was avoided by means of a heating pad and incandescent lamp, respectively. All catheters were tunneled subcutaneously to the interscapular region and there exteriorized. The catheters were filled with a 15% dextran solution and plugged with a short segment of stainless steel wire.

After operation the animals were kept in individual cages and given food and water at will. The condition of each animal was followed by monitoring food and water intake and weight gain.

The experiments were carried out 11 to 19 days after operation, between days 60 and 68 of pregnancy (mean, 62.8 days; term, 68 days). The maternal weights then varied from 860 to 1330 gm. The plan of the experiments is shown in Fig. 1. Maternal blood pressure was recorded continuously on a biomedical strip chart recorder, and heart rate was subsequently counted from the blood pressure record. The dose of isoproterenol used, 0.05 µg · min⁻¹ · kg⁻¹ body weight, was chosen on the basis of a pilot study to give an increase in maternal heart rate of 10% to 15%. The isoproterenol was infused via the jugular vein catheter by means of a constant-speed infusion pump. The dilution was adjusted for an infusion rate of $1 \text{ ml} \cdot h^{-1}$.

Radionuclide-labeled microspheres, 15 µm in diameter (New England Nuclear), were suspended in 0.9% saline solution with 5% dextran and 0.01% Tween

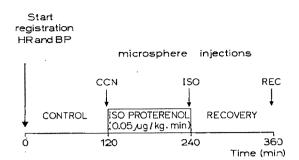


Fig. 1. Diagram of the plan of the experiments. The timing of microsphere injections is indicated by the short arrows. HR, Heart rate; BP, blood pressure; CON, control; ISO, isoproterenol; REC, recovery.

80. The three labels used were scandium 46, tin 113, and cobalt 57. Each microsphere suspension was heated to 38° C and continuously agitated by means of a magnetic stirrer. Each injection consisted of 105 microspheres per 100 gm of maternal weight in an approximate volume of 1 ml. The microspheres were injected via the left ventricular catheter by hand during I minute, and the catheter was flushed with 1 ml of 0.9% saline solution. The reference sample was withdrawn from the femoral artery catheter at a rate of 0.6 ml·min-1 from 30 seconds before the start of the microsphere injection until 90 seconds after the end of the flush.

Approximately 15 minutes after the third microsphere injection the animal was put to death by means of an overdose of sodium pentobarbital. The positions of the catheters were checked. The venous and descending aortic catheters were properly placed in all animals, but in two instances the left ventricular catheter had become displaced into the ascending aorta just distal to the aortic valve. The organs were dissected and prepared for counting as described by Peeters et al.9

was counted with Packard Autogamma Scintillation Spectrometer 5220. In each sample the radioactivity due to each label was calculated with correction for background and overlap. The overlap correction was determined from standard samples of each label. Organ flows were calculated as flow (organ) = flow (reference) × counts (organ) ÷ counts (reference). Cardiac output was taken as the sum of the organ flows. In the two animals in which the left ventricular catheter had become displaced, the coronary circulation contained a disproportionately high number of microspheres; hence the average myocardial flow of the remaining four animals was used in calculating the cardiac output of these two individuals.

Mean arterial blood pressure was calculated from the pulsatile pressure according to the formula (systolic

^{*}p < 0.05 versus preceding value.

Table II. Organ blood flows (ml·min⁻¹·gm⁻¹) before, during, and after infusion of isoproterenol in pregnant guinea pigs

1	1 0	3 1 0	
Organ	Control	Isoproterenol	Recozery
Placenta	347 ± 62	345 ± 76	386 ± ∃3
Myome- trium	18 ± 2	$31 \pm 5*$	31 ± = -
Carcass	19 ± 2	$33 \pm 3*$	21 ± ∃≍
Skin	7.0 ± 0.8	$9.8 \pm 0.7*$	$7.1 \pm 1.0*$
Heart	358 ± 49	653 ± 99	414 ± ∃1 `
(n = 4)	•	•	
Kidneys	588 ± 51	685 ± 63	560 ± ∃ 0
Brain	117 ± 9	$136 \pm 13*$	135 ± 1=†
Gastrointes- tinal tract	176 ± 20	$234 \pm 25*$	176 ± 36*
Pancreas	330 ± 73	481 ± 114*	313 ± 天*
Spleen .	492 ± 119	757 ± 159*	473 ± _=0*
Liver	14.3 ± 2.2	$7.6 \pm 1.2*$	5.3 ± 3.7÷
Mammary gland	49 ± 8	84 ± 14*	42 ± ₹
Lungs	148 ± 19	$201 \pm 38*$	151 ± √I

Values are means ± SEM.

pressure + 2 diastolic pressure) ÷ 3. Total peripr∋ral resistance per 100 gm of body weight was calculated from the mean arterial pressure ÷ cardiac output (ml·min⁻¹·gm⁻¹). Uteroplacental vascular resisance was calculated from the mean arterial pressure ÷ ⊃lacental blood flow (ml·min⁻¹·100 gm⁻¹), thus exaluding myometrial blood flow.

Differences between values were evaluated strustically by the Wilcoxon matched pairs—signed ranks test. Possible correlations were assessed by means of the Spearman rank test.

Results

The average litter size was 3.2 (range, 2 to 4) fet_ses. The mean weight per fetus was 80 gm (range, £_ to 101 gm), and the mean weight per litter was 25± gm (range, 118 to 367 gm).

Infusion of isoproterenol in the near-term pregeant guinea pig produced marked cardiac stimulation (Eable I). Cardiac output increased 41% as a result of increases in both heart rate and stroke volume. Two hours after the end of infusion these measurements had returned approximately to control levels. Mean arterial pressure did not change significantly during the experiments so that calculated total peripheral resistance decreased during infusion of isoproterenol, and returned to control levels during the recovery period (Table I).

Organ blood flows before, during, and after isometerenol infusion are listed in Tables II (ml·min⁻¹ - 100 gm⁻¹) and III (percent of cardiac output). The bood flows to all organs measured, except for the placenta

Table III. Fractions of cardiac output (percent) distributed to various organs before, during, and after infusion of isoproterenol in pregnant guinea pigs

Organ	Control	Isoproterenol	Recovery
Placenta	15.5 ± 1.9	10.9 ± 1.8*	16.5 ± 2.0*
Myome- trium	0.9 ± 0.1	$1.1 \pm 0.1*$	1.5 ± 0.2*†
Carcass	30.8 ± 2.0	$38.1 \pm 2.5*$	$32.4 \pm 3.1*$
Skin	4.0 ± 0.4	4.1 ± 0.3	4.0 ± 0.2
Heart	2.7 ± 0.3	3.4 ± 0.4	2.7 ± 0.1
(n = 4)			
Kidneys	11.5 ± 0.8	$9.5 \pm 0.9*$	$10.8 \pm 0.9 \dagger$
Brain	1.7 ± 0.1	$1.4 \pm 0.1*$	$1.9 \pm 0.2*$
Gastrointes- tinal tract	17.8 ± 1.0	16.7 ± 0.9	17.1 ± 1.2
Pancreas	3.2 ± 0.3	3.3 ± 0.4	$2.8 \pm 0.3*\dagger$
Spleen	2.4 ± 0.4	$2.7 \pm 0.4*$	$2.2 \pm 0.3*†$
Liver	1.7 ± 0.3	$0.6 \pm 0.1*$	$0.6 \pm 0.1 \dagger$
Mammary gland	1.4 ± 0.2	1.7 ± 0.3	1.2 ± 0.2
Lungs	5.2 ± 0.7	4.9 ± 0.8	4.5 ± 0.8

^{*}p < 0.05 versus preceding value.

and liver, increased during isoproterenol. Only the increase in renal blood flow did not reach statistical significance. As noted earlier, myocardial blood flow could be measured satisfactorily in only four animals; but in each case it increased. The ratio myocardial blood flow: heart minute work increased in each animal. The greatest relative increase in flow was exhibited by the carcass, 76%, and this accounted for 56% of the total increase in cardiac output. Increased flow to the gastrointestinal tract accounted for a further 14% of the extra cardiac output during isoproterenol infusion.

Placental blood flow in ml·min⁻¹·100 gm⁻ remained essentially unchanged during isoproterenol, but the placental fraction of cardiac output decreased from 15.5% ($\pm 1.9\%$) to 10.9% ($\pm 1.8\%$). Myometrial blood flow increased 72% during the infusion period, but the proportion of cardiac output distributed to the myometrium increased only slightly. Uteroplacental vascular resistance did not change significantly (Table I).

Two hours after the end of the isoproterenol infusion the blood flows in most organs had returned to near-control levels (Tables II and III). Myometrial and brain blood flows remained significantly elevated at levels similar to those during isoproterenol. Myocardial blood flow, although less than during isoproterenol, was above the control level in three of the four animals in which it could be measured. Hepatic blood flow decreased further during the recovery period, to 37% of the control level.

Placental blood flow increased during the recovery

^{*}p < 0.05 versus preceding value.

[†]p < 0.05 versus control value.

 $[\]dagger p < 0.05$ versus control value.

period, both in ml·min-1·100 gm-1 and as percent of cardiac output. The latter was statistically significant (p < 0.05) in comparison to the value during isoproterenol but not in comparison to the control value.

Comment

The values for maternal heart rate, mean arterial blood pressure, and cardiac output during the control period in these experiments were similar to those found by Peeters et al.9 for chronically catheterized guinea pigs during the same period of pregnancy. With the exception of pancreas, liver, and spleen, the organ blood flows measured were also close to those reported by Peeters et al. The pancreas, spleen, and especially liver flows in the present experiments were notably higher than those observed by Peeters et al.: 330, 492, and 14.3 ml·min⁻¹·100 gm⁻¹ compared with 285, 295, and 2.7 ml·min⁻¹·100 gm⁻¹, respectively. The reason for these differences is not apparent. The control period in the present experiments was longer than that used by Peeters et al., 120 instead of 20 minutes; but if the lower pancreatic, splenic, and hepatic flows found by Peeters et al. were due to catecholamine release caused by the stress of handling during connecting of the catheters, one would expect differences in heart rate, blood pressure, and flows to other organs as well. Feeding behavior during the control period also does not seem to be an adequate explanation, since "it has never been clearly shown that a relationship exists between hepatic arterial flow and hepatic metabolic rate"10

Administration of isoproterenol to the guinea pig in late pregnancy produced cardiac stimulation and an increase in cardiac output that was distributed mainly to the carcass and gastrointestinal tract, these together accounting for 70% of the increment in cardiac output. The balance was distributed generally to all organs measured except the kidneys, liver, and maternal placental vascular bed.

The finding that isoproterenol did not affect placental blood flow or vascular resistance in the guinea pig is in agreement with the results of studies of the adrenergic responses of the uteroplacental vascular bed of sheep.1-6 Intra-arterial injection of isoproterenol in pregnant ewes was found to produce no significant change in either uteroplacental blood flow or vascular conductance,2-4.6 even during the presence of α-adrenergic blockade with phenoxybenzamine.⁵ Small changes in uterine blood flow following intra-arterial administration of isoproterenol to pregnant sheep were interpreted as the consequence of dilatation in extrauterine pelvic vascular beds.6 In pregnant rabbits intra-arterial administration of isoproterenol also did not produce any clear change in placental blood flow as assessed by

radioangiography. 1 A small but significant vasoconstriction in the uteroplacental vascular bed of sheep after an intravenous injection of isoproterenol was probably due to "central reflex responses," that is, adjustments resulting from redistribution of cardiac output to those vascular beds in which vasodilatation was induced. These findings led Greiss^{4, 5} and Greiss and Pick² to conclude that only α-adrenergic receptors were present in the uteroplacental vascular bed of sheep.

Observation of uterine blood flow in nonpregnant sheep and of flow in the uterine artery homolateral to a cotylecon-free uterine horn of a pregnant ewe has indicated that the myoendometrial circulation does exhibit vasodilatation in response to isoproterenol.4.5 This is also the case in the guinea pig, for blood flow in the myometrium increased by 72% during infusion of isoproterenol. Thus in the guinea pig in late pregnancy, as in the sheep, the placental and nonplacental uterine vascular beds differ in their responses to a β-adrenergic stimulation. β-Adrenergic receptors are clearly present in the myoendometrial vessels but cannot be demonstrated by the method we used in the maternal vessels supplying the placenta. Our findings do not permit the conclusion that \(\beta\)-adrenergic receptors are absent from the uteroplacental vessels, however, since these vessels are widely dilated in late pregnancy. Under conditions of maximal or nearly maximal dilatation, a vasodilator may have no detectable effect even though receptors for it are present.

During infusions of the β-receptor agonist salbutamol in pregnant sheep, uterine artery blood flow decreased initially but then returned to control levels 2 hours after the start of the infusion. 12, 13 Since we did not measure uterine artery blood flow continuously and carried out the second microsphere injection after 2 hours of isoproterenol infusion, it is possible that a similar transient reduction in uteroplacental blood flow might have occurred with isoproterenol and not have been detected because of the timing of the flow determinations. The flowmeter studies of Greiss, 1.4.5 Greiss and Pick,2 Ladner et al.,3 and Erkkola et al.6 are not helpful at this point, since only bolus injections were administered in these studies. Additional observations with microsphere injections earlier in the infusion period will be necessary to determine whether uteroplacental blood flow (and indeed other organ flows) remains stable during infusion of isoproterenol. Blood pressure, which was measured continuously in our experimenes, varied minimally during the infusion of isoproterenol, and heart rate increased rapidly to plateau values, which were maintained throughout the infusion

Two hours after termination of the isoproterenol infusion mean placental blood flow had increased about

10% above its level during the control and infusion periods, and myometrial blood flow remained ∈ Evated at the level present at the end of the isopromerenol infusion. These findings are reminiscent of the late increase in uterine artery blood flow observed by Ehrenkranz et al. 12. 14 following 2-hour infusions of f∈noterol and salbutamol and by Brennan et al. 13 aft € about 2 hours of prolonged infusions of these agents in pregnant sheep. It should be noted, however, that the increase in placental flow did not reach statistical significance and that the uteroplacental vascular reatance at this time was not different from that during the control period. Thus there was evidence of variable. tation after the end of the isoproterenol infusing only in the myometrial portion of the uterine vascubiled. The distribution of the increased uterine arter flow was not determined in the studies with salbutarnal and fenoterol.12-14 Our findings in pregnant guinea == s and the observations of Greiss,4.5 comparing the effects of isoproterenol on blood flow to a uterine horn thout placental cotyledons to that to uterine horns with cotyledons, cited above, suggest that the late increase in uterine artery blood flow found with salbutamil and fenoterol¹²⁻¹⁴ was caused by dilatation in the empaplacental úterine vessels.

We wish to thank Mr. G. Grutters, Central ≟nimal Laboratory, Catholic University, and Dr. R. A. M. J. Claessens and Mr. E. B. Koenders, Departmentof Nuclear Medicine, Sint Radboud Hospital, for tL≡ir invaluable technical assistance.

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Glucocorticoids and β-adrenergic—receptor agonists: Their combined effect on fetal rabbit lung surfactant

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In a previous study on pregnant rabbits (AM J Obstet Gynecol 1983;147:437) we found that a prolonged infusion of the β_2 -adrenergic—receptor agonist terbutaline would first cause a release of fetal pulmonary surfactant, so that more was available in the airways. However, the airway fluid then contained less surfactant, indicating a depletion of stores. Since terbutaline is often used in high doses as a tocolytic agent, surfactant depletion could be a serious side effect. With further studies on rabbits, we wanted to test the hypothesis that with an accelerated surfactant synthesis, achieved with glucocorticoids, the increased release, evoked with the terbutaline, would never cause a depletion of the surfactant stores. Our results supported this hypothesis. Betamethasone, administered to the pregnant doe on the twenty-sixth and twenty-seventh days of gestation, 0.1 mg/kg, increased compliance of the fetal lungs, and more phospholipid phosphorus could be lavaged from the airways. These effects were further increased when, following steroid administration, the doe was infused with terbutaline. Depletion of the surfactant stores was never seen when betamethasone was given prior to the β -adrenergic—receptor agonist. (AM J Obstet Gynecol 1985;152:1063-7.)

Key words: Terbutaline, betamethasone, phospholipids, pressure-volume loops of lungs

Inadequate pulmonary surfactant is an important factor in the pathogenesis of the neonatal respiratory distress syndrome (RDS), and early experimental studies demonstrating an ability of glucocorticoids to enhance production and secretion of surfactant held promise for the development of an effective way of preventing RDS. (For a recent review, see reference 1.) The incidence of neonatal RDS in the human is reduced when glucocorticoids are administered to the mother before an anticipated preterm delivery,2 but there is certainly room for further improvement. The β-adrenergic-receptor agonists, frequently used in association with glucocorticoids to arrest premature labor, are of particular interest. Animal experiments by several groups of investigators have made it clear that these drugs, administered to the mother or fetus, will augment secretion of fetal surfactant into the alveolar space.1 In rabbit experiments, carried out on the twenty-eighth day of gestation, we have recently found evidence of increased release of pulmonary surfactant after treatment with terbutaline, a β2-adrenergic-receptor agonist.3 This effect was observed 1 to 6 hours after the drug was first administered, but after a large or long-lasting exposure of the fetuses to terbutaline

we found evidence of depletion of intracellular surfactant stores. Since terbutaline, when used as a tocolytic agent, is often given in massive doses, as large as the mother will tolerate, the possibility that fetal surfactant stores may become depleted must be taken into consideration. It could mean that if delivery occurs at a time when surfactant concentration in airway fluid is lowered by a tocolytic agent, RDS may develop.

With an increased surfactant synthesis, accomplished with glucocorticoids, the β-adrenergic-receptor agonists, although increasing the release of surfactant, might never cause a complete emptying of the stores. A combination of glucocorticoids and β-adrenergic-receptor agonists might therefore result in a higher surfactant concentration in fetal airway fluid without removing all lamellar bodies from the cytoplasm of type II cells. We now report on our studies of the combined effect of betamethasone and terbutaline on the release of pulmonary surfactant into the airways of the fetal rabbit. Lung compliance and total phospholipids of the lung lavage fluid were the parameters assessed.

Material and methods

We used pregnant New Zealand rabbits for which the time of mating was known within 1 hour. On the twenty-sixth and twenty-seventh days of gestation (term 31 days), the doe was injected intramuscularly with betamerhasone (Celestone Soluspan, Schering Corp.), 0.1 mg kg (n = 9), or the same volume of Ringer's solution (n = 8). On the twenty-eighth day, 48 hours after the first injection, the doe was infused intrave-

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Table I. Fetal body weight

Time of infusion	Ringer's solution plus Ringer's solution (gm, mean ± SE)	Ri-⊋er's solution plus &-butaline (_⊋, 21ean ± SE)	Betamethasone plus Ringer's solution (gm, mean ± SE)	Betamethasone plus terbutaline (gm, mean ± SE)
2 hr	35.0 ± 0.6	33.3 ± 0.7	27.2 ± 0.7	25.1 ± 0.9
	(n = 36)	ıп = 29) NS	(n = 39) p < 0.0005	(n = 27) p < 0.0005
				0.025
12 hr			30.7 ± 0.5	26.7 ± 0.4
			(n = 25)	(n = 25)
			p < 0	.0005

Table II. Fetal dry lung weight

Time of infusion	Ringer's solution plus Ringer's solution (mg, mean ± SE)	Riz⊋r': solution plus t∵rbutaline (æ, rıean ± SE)	Betamethasone plus Ringer's solution (mg, mean ± SE)	Eetamethasone plus terbutaline (mg, mean ± SE)
2 hr	109.7 ± 5.3	93.4 ± 3.4	74.7 ± 5.1	53.8 ± 5.5
	(n = 35)	m = 29)	(n = 36)	(n = 25)
		NS	p < 0.0005	p < 0.0005
			p <	0.005
12 hr			88.9 ± 2.8	74.1 ± 2.3
			(n = 24)	(n = 25)
			p < 1	0.0005

nously with terbutaline (Bricanyl, Draco, Swedi -). 20 $\mu g/\min$, for 2 hours (n = 8), or the same volume of Ringer's solution (n = 9). Thus there were four $\pi cups$ of pregnant rabbits, each receiving a specific tope of treatment. Two were first given betamethasone and then received terbutaline (n = 4) or Ringer's scannon (n = 5). The other two groups were injected with R nger's solution rather than betamethasone and were then infused with terbutaline (n = 4) or Ringer's se union (n = 4). Following the 2-hour infusion, the doe v = s put to death with a lethal intravenous dose of thicpertal sodium. Other does received betamethasone in the same way on the twenty-sixth and twenty-sevent days but infusion was for a longer time, for 8 houz with terbutaline, 20 μ g/min (n = 3), or Ringer's sc ution (n = 3). These does were put to death 12 hours after the infusion was begun. As soon as the doe was dead, the abdomen was opened and, to prevent the auses from making inspiratory efforts, they were, while still in utero, injected intracranially with 0.5 ml of the pental sodium. The trachea of each fetus was expos-± and a polyethylene catheter (PE 50) was inserted through an incision just below the larynx. As previously described,3 compliance was then assessed by record ng the pressure-volume relationship simultaneously in all fetuses of a litter. Two consecutive loops were obtained by twice changing pressure from 0 to 10, 20, 11, 35, 30, 20, 10, and back to 0 cm H₂O, with each p≡ssure maintained for 15 seconds. On completion of expliance assessment, the lungs were degassed by rezeated exposure of the entire fetus to vacuum. The lung were then washed with saline solution in a volume in xilliliters equal to one tenth of the body weight in grams. This volume was divided into three equal portions for the same number of lavages. The amount of liquid recovered was 90% to 100% of the saline solution instilled. The lipids of the lung lavage were extracted according to Folch et al.,⁴ and the total phospholipid phosphorus was quantitated according to the method described by Rouser et al.⁵ Following lavage, the lungs were extirpated and dried for 24 hours in an oven at 100° C. This made it possible to report the volume of air entering the lung as milliliters per gram of dry lung. Similarly, the amount of phospholipids, recovered with a lavage, was related to lung tissue examined and expressed as micrograms of phospholipid phosphorus per gram of dry lung.

Student's t test was used for significance evaluation of differences between mean values found in the study. A p value of >0.05 was considered not significant.

Results

Fetal body weight and dry lung weight were significantly lower when the doe had received betamethasone rather than Ringer's solution. This is in agreement with observations made by other investigators. Growth retardation was potentiated when betamethasone treatment was followed by terbutaline infusion. Treatment with terbutaline alone did not affect body weight or dry lung weight (Tables I and II).

Fetal lung expansion improved significantly following betamethasone injection. This effect was further increased when, in addition, the doe was infused with terbutaline for 2 hours. On the other hand, when the

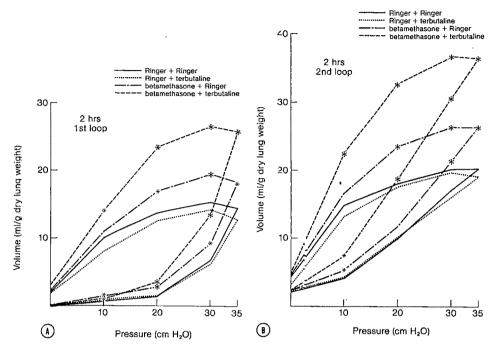


Fig. 1. Pressure-volume loops showing mean volume of air entering the fetal lungs when maternal injections with Ringer's solution or betamethasone were followed by an infusion of the doe with Ringer's solution or terbutaline for 2 hours. (Large asterisk: p < 0.001. Small asterisk: p < 0.05.)

treatment was with terbutaline only, fetal lung expansion did not differ from that of controls (Figs. 1 and 2). The amount of total phospholipid phosphorus of the fetal lung lavage fluid was significantly higher when the doe had been treated with betamethasone and, when terbutaline infusion followed, the effect was even greater (Fig. 3). When the doe had received a prolonged infusion, following a betamethasone injection, the fetuses exposed to terbutaline showed a slight increase in the amount of total phospholipid phosphorus of lung lavage fluid compared to those exposed to Ringer's solution (Fig. 3).

Comment

The synthesis of surfactant in the fetal lung appears to be regulated, at least in part, by circulating corticosteroids. Numerous studies, in vitro and in vivo, have demonstrated that surfactant production is also accelerated by exposure to exogenous glucocorticoids,1 and specific receptors for these hormones have been identified in the fetal lung tissue.7 The mechanism of the glucocorticoid effect is uncertain but apparently includes increased glycogenolysis8 and induction of the following enzymes required for surfactant synthesis: choline phosphotransferase,9 glycerolphosphate phosphatidyltransferase,10 and phosphatidic acid phosphatase.11 The responsiveness of cultured lung tissue to glucocorticoids 12. 13 supports the proposal that glucocorticoids act directly on the fetal lung and exert their effect through the receptor system. Furthermore, Tor-

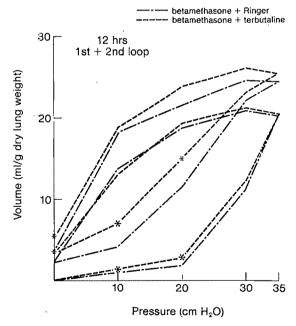


Fig. 2 Pressure-volume loops showing mean volume of air entering the fetal lungs when maternal injections with betamethasone were followed by an infusion of the doe with Ringer's sclution or terbutaline for 8 hours. The doe was put to death 12 hours after initiation of the infusion. (Asterisks as in Fig. 1)

day et al.14 have shown a correlation between the affinity of steroids for lung receptors and their ability to stimulate synthesis of phosphatidylcholine.

The mechanism whereby surfactant appears in fetal

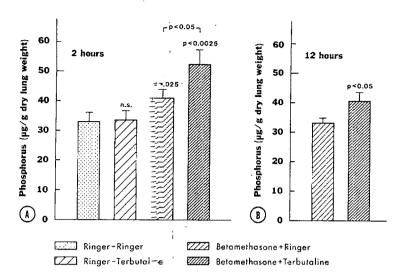


Fig. 3. Phospholipid phosphorus content of fetal lung lavage fluid when maternal injections with Ringer's solution or betamethasone were followed by infusion with Ringer's solution or terbutaline for 2 hours (A). In other experiments maternal injections with betamethasone were followed by infusion with Ringer's solution or terbutaline for 8 hours and the doe was put to death 12 hours after initiation of the infusion (B).

airway fluid also involves β-adrenergic recepto-agonists. These agents are known to cause surfactan. to be released from its site of storage, the cytoplasm of the ype II alveolar cells. Receptors for β-adrenergic-re-cotor agonists have been detected in cell membranes from fetal rabbit lungs, and the number of these recommon increases towards term. In rabbit experiments, Common et al. In noted that this increase would appear procedusly following injection of the doe with betan shasone.

Our study demonstrates improved fetal lung caracity after betamethasone treatment of the doe, and the effect was increased even further when, in addition the doe was infused with terbutaline for 2 or 8 hours. Thout a preceding treatment with betamethasone, the erbutaline infusion, lasting only 2 hours, was unable to effect lung expansion, presumably because the dise was too small. We have previously demonstrated = mat with an infusion given at the same rate but last= 3 8 hours instead of only 2, there were clear indicamens that the surfactant stores had become depleted. Treve pliance was significantly less in the fetuses exposed to terbutaline than it was in the controls. That this cifference was due to a depletion of surfactant store: vas supported by the finding that the lung lavage flu il of terbutaline-exposed fetuses contained significantly less phospholipid phosphorus. In the present study, vien treatment with betamethasone, inducing and stimulating surfactant synthesis, preceded the prolonged_z=rbutaline infusion, we observed no signs of surfactant depletion, yet the terbutaline infusion was identical. It was given at the same gestational age, at the same are,

and the fetuses were examined 12 hours after the terbutaline infusion was begun. The difference was that in the present study the terbutaline infusion was preceded by treatment with betamethasone.

It would seem that when synthesis of surfactant was stimulated by steroids, it was able to keep pace with the accelerated release provoked by terbutaline. Consequently, the surfactant stores never became exhausted. If these experiences from animal experiments can be transferred to the clinical situation, they would imply that when treatment with a β -adrenergic–receptor agonist is combined with attempts to accelerate surfactant synthesis with glucocorticoids, the risk of surfactant depletion and development of RDS is less than if intense tocolysis is attempted with β -adrenergic–receptor agonists not preceded by administration of glucocorticoids.

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Metabolism and disposition of ritodrine in a pregnant baboon

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Relatively little is known about the detailed metabolism of ritodrine. The aim of this study was to examine ritodrine metabolism and pharmacokinetics in the maternal and fetal baboon. A fetal-maternal model was made with use of *Papio anubis* at 144 days of gestation. Tritiated ritodrine was injected as an intravenous bolus into the mother. Maternal and fetal blood samples, amniotic fluid, and maternal unne were collected at timed intervals. Samples were analyzed by a combination of high-pressure liquid chromatography and radiochromatography. Conjugated metabolites were recovered and characterized by cleavage studies with use of β-glucuronidase and sulfatase. The distribution half-life of ritodrine in the mother was 6 minutes and the elimination half-life was 61 minutes. Metabolites were found in both fetal serum and amniotic fluid. The concentrations of ritodrine and its metabolites in fetal serum were at or above the concentrations in maternal serum at 2 hours after maternal intravenous injection. The principal metabolite identified was the sulfate conjugate. The fetus appears to accumulate metabolites. These data indicate that ritodrine crosses the placenta and, in the baboon, achieves levels in the fetus equal to or higher than those in the mother. (AM J OBSTET GYNECOL 1985;152:1067-72.)

Key words: Ritodrine, pharmacokinetics, metabolism

Ritodrine (erythro-p-hydroxy-α-[1-([p-hydroxy-phenethyl]-amino)ethyl]benzyl alcohol) (Fig. 1), a new β-sympathomimetic agent, has been used almost universally for preterm labor patients. Let use first found to be a potent myometrial inhibitor in 1969. Let 1971 ritodrine was first reported to be a well-tolerated uterine relaxant that could arrest preterm labor in most cases. In pharmacokinetic studies employing tritiated ritodrine, Kleinhout and Veth reported that ritodrine

is inactivated by conjugation with glucuronic and sulfuric acids and that the parent compound crossed the placental barrier in a pregnant ewe. In 1980 Gandar et al.⁶ developed a radioimmunoassay method for the determination for ritodrine and reported serum levels and Falf-lives in healthy volunteers. His studies with pregrant women also demonstrated that ritodrine crossed the placenta and entered the fetal circulation. Recently, Nandakumaran et al.⁷ reported a method for the assay for ritodrine by high-pressure liquid chromatography with use of an isolated human placental perfusion model. Their studies also support evidence of ritodrine placental transfer.

Ritodrine is currently the only tocolytic drug approved for obstetric use by the United States Food and Drug Administration Advisory Committee on Fertility

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Fig. 1. Chemical structure of ritodrine.

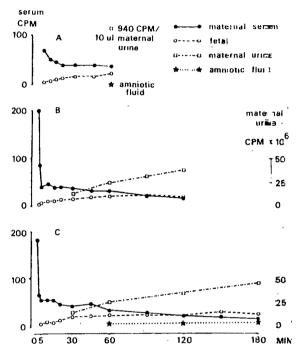


Fig. 2. Total radioactivity of ritodrine and its metabolites rom the maternal and fetal baboon serum, amniotic fluid ≤ 0 μ l sampling), and maternal urine (total output). Data represent first (A), second (B), and third (C) experimental days.

and Maternal Health Drugs.² However, relatively little is known about the detailed metabolism of this crug. The aim of the present study was to examine ritocine metabolism and pharmacokinetics in both the mather and fetal baboon simultaneously.

Material and methods

All reagents used were of analytical grade; solvents were of high-pressure liquid chromatography grede. Both β-glucuronidase and sulfatase (Sigma Chemical Company) were used in the metabolite cleavage experiments. Ritodrine was obtained as its hydrochloide salt from Astra Pharmaceutical Products. A sampe of this material was labeled with tritium by catalytic exchange with use of tritiated water (New England Nuclear, Inc.). The crude tritiated ritodrine was purfied by both thin-layer chromatography and high-pressure liquid chromatography⁸ immediately prior to use.

Serum, urine, and amniotic fluid samples were obtained from a healthy, fertile, female baboon (P-pio

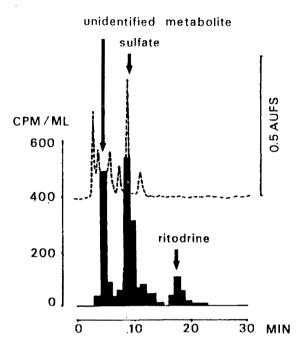


Fig. 3. Ultraviolet and radiochromatogram obtained by highpressure liquid chromatography (solvent programming) of the maternal baboon serum after an intravenous injection of 2.18 μmol of tritiated ritodrine in the mother.

anubis) at a gestational age of 144 days. The baboon was premedicated with ketamine hydrochloride (110 mg) and atropine sulfate (0.6 mg) intramuscularly. The animal was also given hydroxyprogesterone caproate (500 mg) and benzathine penicillin (600,000 U) intramuscularly. The abdomen was shaved, and an intravenous infusion of 5% dextrose in Ringer's lactate was administered in a peripheral arm vein. Electrocardiogram and blood pressure monitoring devices were attached, and the animal was placed in a 15° left lateral position. General anesthesia was administered by endotracheal intubation. The anesthetic consisted of 1% halothane and a 50% mixture of nitrous oxide and oxygen, both at a 300 ml/min flow rate. The abdomen was opened and a small incision made in the anterior myometrium over the area of the fetal lower extremities. Excess amniotic fluid was aspirated, kept warm, and returned to the uterine cavity at the end of the operation. The right lower extremity of the fetus was exteriorized and a cutdown was made in the femoral vein; a fine (PV4) polyvinyl catheter was advanced over an introducer up the vein to a distance of 7 cm (the approximate distance of the renal vessels). The fetal groin incision was closed with a fine polyglycolic acid suture. A fetal electrocardiogram electrode was attached to the right buttock, and a large-bore polyethylene catheter was placed within the uterine cavity to monitor intrauterine pressures and for amniotic fluid sampling. The

amniotic fluid removed earlier was returned to the uterine cavity, and the catheters and electrocardiogram device were brought out through the uterine incision, which was closed in layers with polyglycolic acid suture. Another catheter was placed in the maternal femoral vein for maternal blood sampling. All catheters were filled with heparin (I U/cc). A catheter was also placed in the maternal bladder for urine sampling.

On the day of operation, the first experiment lasted 1 hour. The catheters were then tunneled into the right flank and coiled in a subcutaneous pouch. Total operation time was 100 minutes. Estimated blood loss was 20 ml (baboon blood volume, 65 to 70 ml/kg).9 Fluid replacement consisted of 500 ml of 5% dextrose in Ringer's lactate. After the operation the animal was returned to the cage and received a normal diet enriched in fruit and vegetables, with water ad libitum. The animal's drinking water contained isoxsuprine at a total dose of 20 mg/day.

On the second and fourth postoperative days the animal was returned to surgery after premedication as above. The fetal and intra-amniotic catheters and electrode were exteriorized, and the intra-amniotic catheter and fetal electrode attached to a Corometrics two-channel monitor for evaluation of intrauterine pressure and fetal electrocardiogram.

The second experiment was performed on the second postoperative day. The animal's drinking water on the third postoperative day contained ritodrine at a total dose of 50 mg/day as additional insurance against premature labor. The third experiment was performed on the fourth postoperative day. At the end of each experiment, 4 ml of maternal blood was transfused back to the fetus to restore blood volume. At no time during the experiments did the animal have uterine contractions.

In the experiments a solution (1 ml, 2.18 µmol) of ritodrine hydrochloride containing standard tritiated ritodrine (total activity 2.0 × 108 cpm) was injected as an intravenous bolus into the mother. Maternal and fetal blood samples as well as amniotic fluid and maternal urine were collected at timed intervals.

Blood samples from each experiment were centrifuged immediately. Serum was separated and frozen at -70° C until analysis. The other samples were similarly frozen. An aliquot (10 µl) of each sample was counted for total radioactivity in a Packard Tri-Carb well-type scintillation counter. All samples were then analyzed by high-pressure liquid chromatography.

Serum and amniotic fluid samples were diluted with acetonitrile (1:3 vol/vol), vortexed thoroughly, and centrifuged. The clear supernatant was separated and taken to dryness under nitrogen at 50° C. Each sample

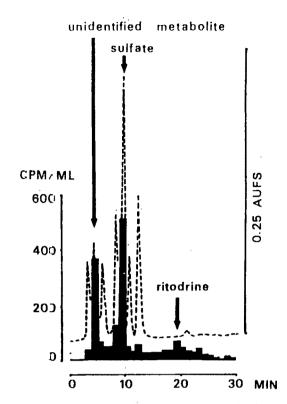


Fig. 4. Ultraviolet and radiochromatogram obtained by highpressure liquid chromatography (solvent programming) of fetal baboon serum after an intravenous injection of 2.18 µmol of tritiated ritodrine in the mother.

was reconstituted with 200 µl of the mobile phase solvent and injected onto the chromatographic column. Urine samples were injected directly onto the column following centrifugation.

Analyses were performed by combined high-pressure liquid chromatography and radiochromatography.8 A Beckman Altex Series 322MP gradient liquid caromatograph, equipped with Beckman Model 165 ultraviolet detector, Gilson fraction collector, and a high-performance phenyl-coated column, were used. The absorption maxima for ritodrine at 225 and 275 rim were used for ultraviolet detection. Ritodrine and metabolite levels in serum, urine, and amniotic fluid were measured radiologically.

Metabolites were recovered from maternal urine for identification. A urine sample (100 ml) was lyophilized, and the solid residues were triturated in methanol. After centrifugation to remove insoluble solids, the supernatant was applied to a conventional liquid chromatography column (30 by 2 cm), packed to a height of 20 cm with silica gel 60. The eluting solvent was a mixture of chloroform/methanol/formic acid (10:5:1 vol/vol), and the sample was eluted under 8 pounds per scuare inch gauge nitrogen pressure. One hundred 4 ml fractions were collected. An aliquot of each was

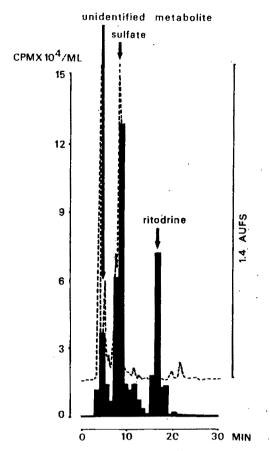


Fig. 5. Ultraviolet and radiochromatogram obtained by Fighpressure liquid chromatography (solvent programming of maternal baboon urine after an intravenous injection of 2.8 µmol of tritiated ritodrine in the mother.

counted for radioactivity. Those with significant activity were examined by high-pressure liquid chromatography, and fractions containing the metabolite were combined for analysis.

A portion of the combined ritodrine metabolite fractions was analyzed directly as a control. A second portion was taken to dryness and reconstituted with water (1 ml). The solution was adjusted to pH 7 (sod mm hydroxide), added to 1000 units of β-glucuronidese, and stirred in a 37° C bath for 3 hours. The reaction was terminated by addition of three volumes of actionitrile and vortexing. After centrifugation and drying of the supernatant under nitrogen, the sample was reconstituted with mobile phase solvent and analyzed by high-pressure liquid chromatography. Fractions (1 =1) were collected and counted for radioactivity.

A third portion was also taken to dryness and reconstituted with 0.4 ml of water. This was added to 10 U of sulfatase along with 0.5 ml of 0.2 mol/L of sodium acetate buffer (pH 5) and 0.1 ml of 0.2% sodium chbride solution. The mixture was incubated as above for 36 hours and then worked up and analyzed as above.

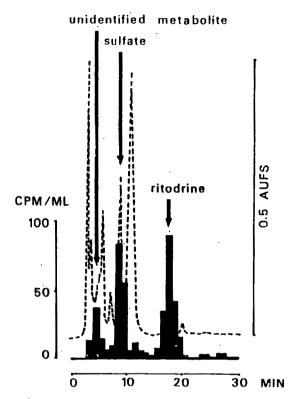


Fig. 6. Ultraviolet and radiochromatogram obtained by high-pressure liquid chromatography (solvent programming) of baboon amniotic fluid after an intravenous injection of $2.18~\mu mol$ of tritiated ritodrine in the mother.

Results and comment

The total radioactivity plotted against time for the three experiments (A, B, and C in Fig. 2) showed maternal serum total radioactivity fell rapidly in the first 5 minutes and much more slowly after that. The fetal pattern was different: in all three experiments the total radioactivity rose slowly over 30 minutes and remained essentially constant thereafter. In the third experiment (Fig. 2, C) fetal levels exceeded those of the mother after 2 hours.

This confirmed that ritodrine and/or its metabolites cross the placenta to the fetal circulation. Kleinhout, in his study of the pregnant ewe,⁵ reported that the concentration of ritodrine in the fetus was only 20% of that of the mother 4 hours after a 20-minute infusion of the drug. Our results would seem to indicate that levels in the baboon fetus quickly reach or exceed those in the mother. One may speculate that there are interspecies differences in ritodrine placental transfer.

Total urinary radioactivity output from the mother increased over the period of collection in all three experiments. Total activity in the amniotic fluid remained at a constant level, slightly lower than that of fetal serum, over the 3 hours of the third experiment. Hence fetal elimination of ritodrine and its metabolites ap-

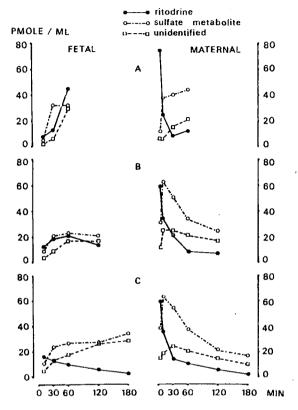


Fig. 7. Fetal and maternal baboon serum levels of ritodrine and its metabolites after an intravenous injection of 2.18 µmol of tritiated ritodrine in a pregnant baboon, from first (A), second (B), and third (C) experimental days.

pears to be very slow in comparison to that of the mother.

When the samples were individually examined by high-pressure liquid chromatography, three separate peaks of radioactivity were found, which corresponded to ritodrine and two metabolites. There is a quantitative similarity in the maternal and fetal serum samples (Figs. 3 and 4) and in the maternal urine and amniotic fluid (Figs. 5 and 6), with the latter showing much higher relative ratios of parent drug than the former. This would seem to be due to urinary output of the fetus into the amniotic fluid.

The identity of the early-eluting metabolite has not yet been determined, although its extremely short retention time indicates a highly polar compound; there is no firm evidence for its structure at this time, although in other experiments in this laboratory it has been demonstrated that ritodrine is capable of undergoing phase 1 metabolism in the rat with cleavage of the secondary amino group. The second metabolite was positively identified as the sulfate conjugate of ritodrine. Fig. 7 summarizes the results of those experiments.

From a semilog graph of maternal ritodrine serum

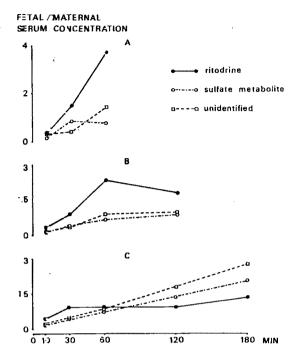


Fig. 8. Ratio of the concentration of ritodrine and its metabolites in he fetal serum to maternal serum, from first (A), second (E), and third (C) experimental days.

concentration versus time, a distribution half-life of 6 ± 1 minute and an elimination half-life of 61 ± 1 minute vere determined. The elimination half-life was about half that found by Gandar⁶ in nonpregnant human female volunteers receiving a 1-hour constant-rate ritodrin≥ infusion.

In the fetus the serum concentration pattern of ritodrine varies among the three experiments. In the first two experiments the ritodrine levels in the serum increased up to I hour and then declined, but in the third the levels decreased continuously from 10 minutes to 3 hours. This was obviously due to the ritodrine the mother had been receiving in her drinking water on the day previous to the experiment. Fig. 8 summarizes the calculated fetal/maternal serum concentration ratics for the three experiments.

The naternal serum showed a very rapid rise of both metabo ites within the first 10 minutes (Fig. 7), with the sulfate conjugate as the predominant compound. In the second and third experiments the rate of elimination of all three compounds appeared approximately equal.

Both metabolites were also found in the fetal serum, although they did not show the initial surge seen in the mother. Rather, their concentrations increased slowly, achieving levels comparable to those of the mother after 60 min_ites. Their continued slow build-up, as seen in the third experiment, suggests that the fetus is at a disadvantage relative to the mother in disposing of these compounds and that they can accumulate in the fetal circulation even though the parent drug is leared, perhaps because of a poor recrossing of the pacental barrier.

The enzymatic degradation studies of the metabolite isolated from the maternal urine showed that it was inert toward β -glucuronidase but was converted in high yield to the parent drug by sulfatase. Thus the only isolable conjugate of ritodrine in the baboon was the sulfate ester.

Ritodrine administered to the pregnant babcon can cross the placenta and achieve levels in the fetal circulation equal to or higher than levels in the mother. The fetus appears to accumulate the metabo tes to some extent. It would seem reasonable to assume that any extrauterine effects of ritodrine caused by sympathomimetic activity should therefore be expected to be seen in the fetus as well as the mother.

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Initiation of angiogenesis by porcine follicular fluid

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Angiogenesis was observed and measured after injection of porcine follicular fluid into rabbit corneas. A qualitative response (0 to 6+) and quantitative measurement (mm/day) were obtained 9 days after injection. Undiluted porcine follicular fluid stimulated angiogenesis with new blood vessels visible by the third day after injection, extending 2.0 to 3.0 mm into the site of injection from the corneal scleral limbus (1 to 4+) by day 9. Angiogenic activity was consistently found in fractions of porc ne follicular fluid which precipitated in 20% to 40% saturated ammonium sulfate. Sephadex gel filtration of the 20% to 40% saturated ammonium sulfate fraction resulted in fractions with molecular weights of 45,000 to 60,000 and ≤1500 daltons which stimulated angiogenesis. Charcoal treatment of active fractions did not remove angiogenic activity. Angiogenic activity was retained after heating at 56° C for 1 hour but was lost after boiling (20 minutes). Quantitative measurements of chemotaxis with use of Boyden chambers and mitogenesis by means of tritiated thymidine incorporation were performed. Follicular fluid from small follicles contained greater chemotactic activity than follicular fluid from medium or large follicles. The 20% to 40% saturated ammonium sulfate precipitate that eluted through Sephadex G-100 with a molecular weight of 45,000 to 60,000 daltons contained angiogenic, mitogenic, and chemotactic activity. In conclusion, porcine follicular fluid contains angiogenic factors that may be associated with perifollicular neovascularization during folliculogenesis. (AM J OBSTET GYNECOL 1985;152:1)73-8.)

Key words: Angiogenesis, follicular fluid

Angiogenesis, the growth of new blood vessels, plays a major role in a variety of important biologic processes, including wound healing, 1-3 tumor growth, 1-6 and embryonic development. Angiogenesis also occurs during maturation of the preovulatory follicle 7-10 and subsequent formation of the corpus luteum, 10-12 which suggests that the developing follicle and/or its surrounding theca may produce an angiogenic factor. Recently we reported the presence of angiogenic activity in ovarian follicular fluid aspirated from women participating in an in vitro fertilization protocol. 13

Two aspects of studies relating to angiogenesis should be kept in mind: (1) new blood vessel growth requires endothelial cell replication and migration; (2) nonspecific inflammation and thus overinterpretation of experimental data may result from manipulations of the rabbit cornea. Accordingly, angiogenic factors must be able to stimulate chemotaxis and cell proliferation in addition to initiating new blood vessel growth in the absence of inflammation. With these caveats in mind,

we wish to report further studies on angiogenic activity in follicular fluid. These studies are an extension of our previous report describing angiogenic activity in human follicular fluid. Here we used fresh porcine follicular fluid because of its ready availability.

Material and methods

Porcine follicular fluid was aspirated from follicles present in fresh ovaries obtained at a local abattoir. Initially, whole follicular fluid from medium-sized follicles (3 to 5 mm in diameter) was precipitated by the serial addition of saturated ammonium sulfate (20%, 40%, and 50%) followed by a 2-hour equilibration period. The precipitates were concentrated by centrifugation (5000 \times g, 20 minutes) and the supernatant decanted. The pellets were resuspended in buffer A (Tris-HCl, pH 7.5), dialyzed against distilled water (2 volumes, 4° C, 24 hours) and lyophilized.

The ammonium sulfate precipitates were further separated by elution through a Sephadex G-100 column (400 × 4 cm; Pharmacia, Piscataway, New Jersey) with buffer A. Fractions were collected and absorbance of the eluent (280 nmol/L) determined by an Isco absorbance detector (Lincoln, Nebraska). Before testing, pooled chromatography fractions were dialyzed against distilled water (36 hours, 70° C) and lyophilized. The lyophilized material was resuspended in 0.5 to 1.0 ml of buffer A before testing in the corneal implant assay. Apparent molecular weights were estimated by column

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QUALITATIVE GRADE

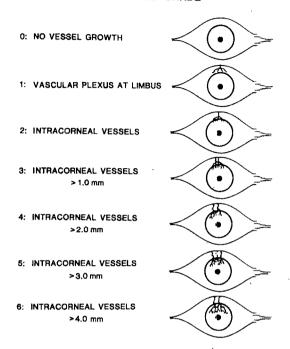


Fig. 1. Qualitative grading system used to evaluate the = Tects of follicular fluid on angiogenesis.

calibration with molecular weight standards (Fharmacia).

Additional separation was performed using — Amicon molecular weight sieve (Amicon, Lexingto1, Massachusetts). The ammonium sulfate precipitates were suspended in buffer A and then processed the 12h a PM 30 membrane (molecular weight cutoff of 100) in an Amicon pressurized chamber (5 psi). The fialysate was further processed through a PM 10 membrane in a similar fashion. All follicular fluid fractions were lyophilized before testing in the rabbit corneal ==55.

Angiogenesis assay. Before analysis, follicul__fuid was centrifuged (600 × g, 30 minutes) to remer ≡ cellular debris. Each follicular fluid test sample was mixed with an equal volume of a Hydron solution (1-7-thon, New Brunswick, New Jersey) containing 10% I-thon, 60% ethanol, and 1% polyethylene glycol (mclecular weight = 400). The effect of the follicular flux—Hydron mixture on angiogenesis was assessed in N=> Zealand White female rabbits (1.5 to 2.0 kg). The rabbits were anesthetized with ketamine hydrochloride 23 mg/ kg, intravenously) and then a 20 μl aliquot of = follicular fluid-Hydron solution was injected into the right cornea by aseptically creating a pocket 1 mm moximal to the superior limbus. 13, 14 The left cornea ≡1 ≥ach animal was injected with Hydron and saline saline (control). The corneas were evaluated daily for I days after implantation. Sustained growth of well-4=fined new capillaries from the limbus toward or into the corneal implant was considered positive for angiogenesis. A qualitative response was scored on a relative scale as follows: 0 = no angiogenesis; 1 + = plexus of blood vessels seen at limbus; 2 + = intracorneal vessels; 3 + = intracorneal vessels > 1.0 mm from limbus toward injection site; 4 + = intracorneal vessels > 2.0 mm from limbus toward injection site; 5 + = intracorneal vessels > 3.0 mm; 6 + = intracorneal vessels > 4.0 mm (Fig. 1). Quantitative measurements of vessel growth (mm/day) were also obtained as previously described.¹³

Thermolability of the angiogenic activity was determined by allowing whole follicular fluid as well as separated active fractions to stand overnight at room temperature (26° C) and then heating for 1 hour at 56° C or boiling for 20 minutes. These aliquots were centrifuged (5000 \times g, 30 minutes) and the supernatants lyophilized before corneal assay. Aliquots containing angiogenic activity underwent charcoal stripping (1:1 vol/vol, 0.5% Norite A, 18 hours, 6° C). The supernatant was collected by centrifugation (3000 \times g, 15 minutes), passed through a sterile filter (22 μ m, Amicon), and dialyzed against distilled water. Retentates were lyophilized prior to testing in the corneal implant assay.

Corneas supporting angiogenic activity were fixed in formalin, serially sectioned and stained with hematoxylin and eosin for histologic evaluations to control for nonspecific inflammation.¹³

Endothelial cell preparation. The procedures for obtaining and maintaining cultures of fetal aortic endothelial cells have been previously described in detail. Minimum essential medium (Grand Island Biological, Grand Island, New York) containing Earle's salts, penicillin (200 U/ml), streptomycin (200 μg/ml), glutamine (2 mmol/L, and fetal calf serum (10%; Sterile Systems, Logan, Utah) was employed as the growth medium. Subculturing was routinely performed every 2 days with use of split ratios of cells (1:3 to 1:5). Endothelial cells from passages 10 to 18 were used for testing purposes.

Mitogenesis assay. Mitogenic assays were carried out in 24-well plastic dishes (Falcon Plastics, Oxnard, California). With use of these conditions, cell viability of >95% was usually obtained by hemocytometer measurements of cells suspended in a trypan blue solution. Cells resuspended in the growth medium were plated in the multiwell dishes (10⁴ cells per well); 16 hours later the attached cells (approximately 60% to 75% of the original plant) were rinsed with calcium- and magnesium-free phosphate-buffered saline solution. Test materials consisted of follicular fluid fractions (100 µl) in serum-free media 199 (1 ml per well). Replicate wells in the same dish were employed for each test condition. The actual number of cells present at the start of the

test were measured by detaching the cells from several wells with 0.1% trypsin (type III from bovine pancreas, Sigma, St. Louis) and 0.05% ethylenediaminetetra-acetate, and cell counts were performed. Attachment efficiencies of ~65% have been obtained in our laboratory after an overnight (16 hours) incubation in growth medium.

Test cultures were incubated at 37° C in a humidified 5% carbon dioxide atmosphere for the specified period of time. Incorporation of tritiated deoxythymidine was performed by the following pulse-labeling technique: I hour before the end of the experiment, the test media was removed and replaced with 1.0 ml per well of serum-free media 199 containing 0.625 µCi/ml of [3H] deoxythymidine (Amersham, Arlington Heights, Illinois) as a sterile, aqueous solution (20 to 25 Ci/mmol, diluted to 6.7 Ci/mmol and used within 2 months). After a 1-hour incubation at 37° C, the incorporation was terminated by removing the culture fluid and rinsing each well once with cold (4° C) calcium- and magnesium-free phosphate-buffered saline solution and 50% acetic acid-ethanol (1 part glacial acetic acid and 3 parts 80% ethanol). These cells were then extracted with the acetic acid-ethanol solution. After fixation, the cells were reextracted with 0.3N perchloric acid for 10 minutes and then thoroughly rinsed with Dulbecco's phosphate-buffered saline solution (pH 7.4, 0.2 mol/ L). Solubilization of cellular materials was accomplished by incubating the contents of a well with 0.2 ml of 0.25N sodium hydroxide solution for 20 minutes at room temperature. The contents of each well was transferred to 4 ml glass counting vials with use of an additional 0.2 ml of water for rinsing the well. Each vial then received 3.2 ml of scintillation fluid, the contents were mixed, and the vials were counted in a scintillation counter. Fractions containing mitotic activity were assayed by means of triplicate determinations.

Chemotaxis. The method is based on the passage of cells across pores against a gradient of a chemotactic affector.15 The Boyden chamber used had an upper well of 200 µl and a lower well of 40 µl. The two wells were separated by a polyvinylpyrrolidone nucleopore filter (Nucleopore, Pleasanton, California), 13 mm in diameter and precoated with gelatin. An 8 µmol/L micropore filter was used for fetal aortic endothelial cells. Both chambers were filled with Medium 199 added with 10% fetal calf serum. About 105 cells in 40 µl of medium were placed in the upper chamber, and the follicular fluid test fraction was located in the lower chamber, containing 40 µl of Dulbecco's modified Eagle's medium plus 10% fetal calf serum. Incubation (37° C, 5% carbon dioxide) lasted for 3 hours, which was sufficient to obtain good migration. At the end of incubation the cells on the upper surface of the filter were

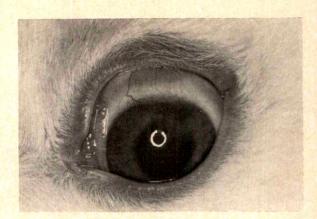


Fig. 2. Growth of new blood vessels in rabbit cornea after injection of a dialyzed 20% to 40% saturated ammonium sulfate precipitate of porcine follicular fluid eluted through a Sephadex G-100 column.

removed with a cotton swab. The cells that had migrated to the lower filter surface were fixed in 10% formalin in buffered phosphate, stained with Wright's stain, and counted on five different low-power fields for each filter. Each determination was performed in triplicate. Tests for statistical significance in all assays were performed by Student's t test.

Results

Undiluted follicular fluid stimulated angiogenesis in four out of four rabbits. The invasion of blood vessels was macroscopically visible by the third day after injection, and by day 9 had extended 3.0 mm into the site of injection from the closest region of the cornealscleral limbus (Fig. 2).

All the dialyzed and lyophilized fractions of the 20% to 40% saturated ammonium sulfate pellet (4/4) demonstrated 3+ angiogenic activity in the rabbit corneal implant assay 10 days after injection. The other fractions (the 0% to 20% precipitate and the 40% to 50% supernatant) produced negative or inconsistent results. Accordingly, all further analyses were performed with use of the dialyzed 20% to 40% saturated ammonium sulfate pellet as starting material.

The angiogenic activity in fractions from a representative Sephadex G-100 chromatogram of the 20% to 40% pellet is shown in Fig. 3. Fractions containing angiogenic activity corresponded to molecular weight ranges of 45,000 to 60,000 daltons and ≤1500 daltons. These dialyzed follicular fluid fractions induced angiogenesis with vascular growth extending up to 2.5 mm (4+ grade) into the site of injection by day 9. The PM 10 dialysate contained activity in all fractions tested (4/ 4), whereas the PM 30 dialysate and PM 10 retentate did not induce angiogenesis.

Although angiogenic activity was lost after boiling (20

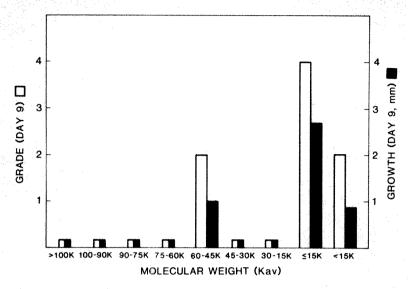


Fig. 3. Absorbance pattern of a dialyzed 20% to 40% saturated ammonium sulfate precipitate of porcine follicular fluid which eluted through a Sephadex G-100 column. Growth of new blood vessels in rabbit cornea occurred after injection of fractions which eluted in the molecular weight range of 45,000 to 60,000 and <1500 daltons.

minutes) of the 20% to 40% saturated ammonium sulfate pellet and PM 30 retentate, no apparent loss in activity of these fractions was observed after heating at 56° C for 1 hour (3+, day 9) or after standing at room temperature (2+, day 9). Charcoal-stripped follicular fluid stimulated angiogenesis with a plexus of blood vessels growing up to 2.0 mm by day 9 (Fig. 4). Left eyes were injected with Hydron and saline solution and consistently showed no angiogenic activity. The occasional rabbits that demonstrated a response (<5%) were excluded from the study. Every eighth cornea considered positive for angiogenesis was evaluated histologically. None contained significant evidence of a polymorphonuclear infiltrate.

Mitogenic assay. All whole porcine follicular fluids tested contained mitogenic activity compared to bovine serum albumin (10 mg/100 ml, body surface area) controls (small follicle, 247% ± 29%; medium, 189% ± 18%; large, $178\% \pm 21\%$ of total counts per minute incorporated per milligram of protein). Again, the 20% to 40% saturated ammonium sulfate "cut" contained significant mitogenic activity in the thymidine incorporation assay (184% \pm 51%, p < 0.05) while the follicular fluid fraction in the 20% and 50% to 100% saturated ammonium sulfate precipitates contained no increase in tritiated thymidine incorporation (101% ± 18% and 93% \pm 27%, respectively). The 40% to 50% follicular fluid saturated ammonium sulfate fraction induced a trend toward increased mitogenic activity $(184\% \pm 87\%)$ which was not significantly different from body surface area control values.

The Sephadex G-100 fraction corresponding to

45,000 to 60,000 daltons contained nondializable material that significantly enhanced mitogenesis (341% \pm 41%, p < 0.05). In addition, the dialysates of the G-100 fraction corresponding to <10,000 daltons stimulated thymidine incorporation (287% \pm 34%, p < 0.1).

Chemotaxis assay. Whole follicular fluid (100 µl) from small (<2 to 3 mm) follicles induced significantly more fetal aortic endothelial cell migration (521% ± 47%) compared to medium (3 to 5 mm diameter) or large follicles (>5 mm diameter) (191% ± 18% and $164\% \pm 47\%$, respectively). The only consistent ammonium sulfate fraction which after dialysis facilitated chemotaxis was the 20% to 40% "cut," irrespective of follicle size tested (254% ± 31% of buffer control value). Specifically the 20% saturated ammonium sulfate precipitate and 40% saturated ammonium sulfate supernatant of porcine follicular fluid did not reproducibly stimulate chemotaxis after removal of the ammonium sulfate by dialysis. This of course does not exclude the possibility that another fraction of follicular fluid having a solubility different from that of the 20% to 40% saturated ammonium sulfate fraction contained chemotactic activity, since that material might have been removed with dialysis. In addition, the ammonium sulfate containing dialysates may have obscured any chemotactic activity. Pooled follicular fluid that eluted at 45,000 to 60,000 daltons contained chemotactic activity (100 µl containing 600 µg of protein: $378\% \pm 48\%$ of buffer control). Most of the other chromatographic fractions also contained chemotactic activity, albeit significantly less than the chemotactic activity in the 45,000 to 60,000 dalton fraction. Because

of the need to dialyze chromatography fractions to equilibrate salt concentrations before Boyden chamber testing, follicular fluid fractions < 10,000 dalton could not be reliably tested for chemotaxis.

Comment

The results reported here suggest the presence of angiogenic activity (including chemotaxis and mitogenesis) in porcine follicular fluid that was extractable with 20% to 40% saturated ammonium sulfate. This activity was demonstrated in two distinct molecular weight ranges: 45,000 to 60,000 daltons and ≤1500 daltons. Charcoal stripping did not remove the angiogenic activity. Although boiling inactivated angiogenic activity, heating at 56° C for 1 hour did not appear to remove angiogenic factor(s).

What might be the function of angiogenic factors in follicular fluid? During the follicular phase of the human menstrual cycle, a single follicle usually matures and ovulates in response to the midcycle surge of serum gonadotropins. The question arises as to why only a single follicle develops to maturity, while others undergo atresia in the same gonadotropin environment. Previously diZerega and Hodgen⁸ and Zeleznik et al.⁹ demonstrated an increasing density of blood vessels within the follicle complex of the primate dominant follicle. This increase in perifollicular vascularization may result in preferential gonadotropin delivery and thus in selection and maintenance of the dominant follicle. That greater chemotactic and mitogenic activity was found here in small porcine follicles suggests the presence of differential mitogenic activity during fol-

Basset⁷ described the appearance of perifollicular blood vessels during growth and regression of rat follicles. From the endothelial walls of these vessels, angiogenic sprouts begin to grow into the granulosa layer associated with a rapid increase in capillary permeability. Previously, Jakob et al. 12 reported that the rat corpus luteum produced a vigorous stimulation of capillary growth. In 1983 Koos and LeMaire¹⁰ described the induction by rat follicles and corpora lutea of gonadotropin-responsive angiogenesis with use of the chorioallantoic membrane of the chick embryo. Gospodarowicz and Thakral¹¹ found that explants of rabbit corpora lutea stimulated angiogenesis when placed into a rabbit cornea. Recently Frederick et al. 13 reported the presence of angiogenic activity in human follicular fluid. Taken together, these studies suggest that a signal for angiogenesis accompanies follicular development and formation of the corpus luteum in a variety of mammalian species.

Our findings are consistent with those of Makris et al.16 who reported that angiogenic activity appears

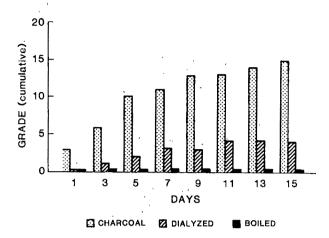


Fig. 4. Cumulative growth (by grade) of new blood vessels in rabbit cornea after injection of a dialyzed 20% to 40% saturated ammonium sulfate precipitate of porcine follicular fluid eluted through a Sephadex G-100 column (molecular weight of 45,000 to 60,000 caltons) after charcoal stripping, dialysis, or boiling.

to have some stability to moderate heat (56° C) but not to boiling. D'Amore et al.5, 17 and Auerbach reported angiogenic activity associated with both high and low molecular weight isolates from a variety of starting materials including turnors and retina. Our results are also consistent with these findings. Angiogenic activity was found in both Sephadex and Amicon molecular sieving procedures in fractions with molecular weights corresponding to 60,000 to 45,000 daltons and ≤1500 daltons. Unfortunately, reliable mitogenic and chemotactic assays could not be performed on the <10,000 dalton fraction of follicular fluid for technical reasons. The possibility that the active angiogenic material could be a small molecule, linked to a larger carrier molecule, was suggested by D'Amore et al.17 and Banda et al.3 Other explanations for these observations include (1) heterogeneity of active material, (2) molecular aggregation, and (3) artificial fragmentation of a large (>45,000 daltons) molecule. Others have reported angiogenic activity in purified fractions with a molecular weight of ≤200 daltons from rabbit wound fluid,5 cat retina,5 and Walker 256 tumor.6.14 Taken together, these findings are consistent with a low molecular weight angiogenic factor that combines with a higher molecular weight carrier protein. Similar observations of an angiogenic activity in high (45,000 to 60,000 daltons) and low (≤1500 daltons) molecular weight fractions have been made in purified human follicular fluid specimens (Frederick, J. L., and Preston, D. S., unpublished results). These observations lead to speculation that control of argiogenic activity outside the basal lamina of the follicle may occur by alterations in carrier protein association with angiogenic factor(s). In conclusion, our data suggest that, in porcine follicular fluid, there is material that stimulates angiogenic activity in the rabbit corneal implant assay.

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Treatment of Postmenopausal Women With Transdermal Delivery of Estradiol

The following papers are selected from presentations given at the symposium "Treatment of Postmenopausa. Women With Transdermal Delivery of Estradiol," held at the Fourth International Congress on the Menopause, in Orlando, Florida, on Nov. 1, 1984.

This symposium was supported by a grant from CIBA Pharmaceutical Company.

Endometrial responses to transdermal estradiol in postmenopausal women

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In prospective studies, we have determined the endometrial histologic characteristics and patterns of vaginal bleeding in 12 perimenopausal or postmenopausal women during administration of transdermal estradiol, 0.05 mg daily, given either alone or in combination with a progestogen. In the first study, we administered transdermal estradiol in cyclical fashion for 3 months. Outpatient curettage at pretreatment produced no endometrial sample or tissue too scant for assessment from 10 of the 12 patients (83%). At the end of therapy, proliferative or nonsecretory endometrium was diagnosed in nine patients (75%). Eight patients experienced treatment-related vaginal bleeding but no regular pattern, and seven patients reported breakthrough bleeding. Eight patients participated in the second study in which transdermal estradiol was administered continuously and norethindrone, 0.35 mg, was added for 12 days of each calendar month. A further curettage was performed at the end of treatment, and proliferative endometrium was the most common finding. No endometrial hyperplasia was observed. Only one patient experienced breakthrough bleeding. There were no consistent changes with time in the number of patients bleeding each month or in the duration or heaviness of the bleeding. (AM J OBSTET G~NECOL 1985;152:1079-84.)

Key words: Transdermal estradiol, endometrial histology, uterine bleeding

At present, the major hazard of exogenous estrogen therapy is the genesis of endometrial hyperplasia and carcinoma.¹⁻³ Additionally, marked endometrial proliferation during cyclical estrogen therapy may lead to unscheduled vaginal bleeding,¹⁻² which is a potent cause of anxiety for both the clinician and the patient. Irregular bleeding requires appropriate invasive procedures that have well-recognized implications in terms of morbidity and cost.

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Reprint requests: M. I. Whitehead, Senior Lecturer, Academic Department of Obstetrics and Gynaecology, King's College School of Medicine and Dentistry, Denmark Hill, London SE5 8RX, England. With oral estrogens, a proportion of the administered steroid is initially metabolized within the gut wall, and estradiol is preferentially converted to estrone. It is then delivered by the portal circulation to the liver, where further metabolism occurs and estrone undergoes conversion to estrone sulfate and estrone glucuronide. Neither of these metabolites is believed to be physiologically active. It has been estimated that up to one third of the total administered dose of estrogen is metabolized in these ways before the systemic circulation is reached. Thus, oral estradiol therapy results in a relatively small increase in plasma levels of estradiol, a large increase in plasma concentrations of estrone, and the development of a "pool" of circulating estrone sulfate and estrone glucuronide. 1.5.6

Plasma estradiol values correlate more strongly than does estrone with the occurrence of flushing and sweating episodes and with vaginal atrophy. Thus, to achieve therapeutic plasma concentrations of estradiol, oral estrogens have to be given in large doses which give rise to supraphysiologic concentrations of ∋strone and to nonphysiologic pools of estrone sulfate and estrone glucuronide. 5.6

Large circulating concentrations of estrone, estrone sulfate, and estrone glucuronide may have undesirable effects upon the endometrium. Estrone possesses biologic activity, and, after withdrawal of exogenous estrogen therapy, estrone sulfate may be bak-converted to estrone, thereby potentially maintaining a stimulus for continued endometrial proliferation. We estimate that, with oral estrogen therapy, the plasma levels of estrone sulfate are sufficiently high for back-conversion to continue for many days. Therefore, the endometrium may have little respite from stimulation in the treatment-free week of cyclical therapy.

As compared to oral therapy, transdermal escradiol delivers a low daily dose of estrogen and does not increase plasma levels of estrone and estrone sullate to the same degree. Transdermal administration of estradiol provides plasma levels sufficient to relieve menopausal symptoms, and back-conversion after withdrawal of therapy is likely to be less marked and less prolonged.

We wondered whether transdermal estradiol would induce marked endometrial proliferation and cause vaginal bleeding. Therefore, we conducted a str.dy of the endometrial effects of transdermal estradiol Estraderm TTS, CIBA-GEIGY Ltd., Basel, Switzer and), 0.05 mg daily. The study consisted of two phases. The first phase lasted 13 weeks, during which transdermal estradiol was administered for 3 out of 4 weeks, with 1 intervening treatment-free week (cyclical therapy); the second phase lasted for 6 months and involved daily administration of transdermal estradiol with the addition of a progestogen for the first 12 days of each calendar month (continuous-sequential therapy... We performed endometrial biopsies at the end of each treatment phase and examined the tissue by light microscopy. We also monitored the occurrence of all vaginal bleeding.

Patients and methods

This study was an open trial that involved pazients who attended the Menopause Clinic at King's College Hospital, all of whom gave informed consent. Trelve patients participated in Phase 1, and eight of these participated in Phase 2 of the study. The other four patients finished Phase 1 of the study but could not continue into Phase 2 because no more transdermal estra-

dio systems were available at that time. (These four patents were transferred to other forms of exogenous estengen therapy.) At the commencement of Phase 1, all 2 subjects had to be at least 6 months postmenopausal, with a plasma level of follicle-stimulating hormone (FSH) within the postmenopausal range. All patients were experiencing menopausal symptoms, and note had received estrogen therapy in the 3 months prict to recruitment.

Pase 1. Phase 1 lasted 13 weeks and involved three visits to the Clinic. The first visit was a pretreatment assesment during which venipuncture was performed for measurements of plasma FSH, luteinizing hormone (LH), estradiol, and estrone. The second visit occurred 2 weeks after the first visit, and, if the plasma level of FSE determined at the first visit was within the postmerpausal range, the patient was recruited for the stud and an outpatient curettage was performed. The tran-dermal estradiol patches were prescribed for 3 weeLs, followed by 1 treatment-free week; this sequerce was repeated three times. Each patch was design-d to deliver 0.05 mg of estradiol per day, and each patca was applied for 3 days. Patients were advised not to stick the patches near their breasts or genitalia but to uze sites such as the abdomen, buttocks, or thighs. The patches were applied after bathing and drying. Once the patch was in situ, it was not removed under any arcumstances until completion of the 3-day period (patients bathed and showered with the patches in place). Further details on the patients can be found in the mort of Padwick et al.11 The third visit occurred during the third treatment week of the third cycle, when a second curettage was performed.

Phase 2. Phase 2 lasted 6 months and involved two visits At the first visit the new treatment regimen was explained. The estradiol patches were administered continuously for 6 months. The patches were identical to those used in Phase 1 and, again, were replaced twice week. In addition, norethindrone, 0.35 mg (Micronor, Orth. Pharmaceutical, Raritan, New Jersey), was prescribed daily for the first 12 days of each calendar montin. Previous studies have suggested that this small dose of norethindrone may protect against the development of endometrial hyperplasia. 12

The second visit occurred in the last week of the sixth monta of therapy, approximately 2 weeks after the sixth course of norethindrone, when a further endometrial biopsy specimen was taken. This was the time of maximal unopposed estrogenic stimulation.

Assessments.

Encocrine. Plasma levels of FSH, LH, estradiol, and estrone were analyzed by CIBA-GEIGY, Basel, Switzerland, as described previously.

Endometrial biopsies. Vabra suction curettage13 was per-

formed to obtain endometrial tissue, which was immediately fixed in formol-acetic acid and assessed by light microscopy.

Bleeding charts. A daily record of all bleeding that occurred throughout Phases I and 2 was kept by every patient. With cyclical therapy, withdrawal bleeding was defined as bleeding that occurred during a treatmentfree week; breakthrough bleeding was defined as that which occurred at any other time. With continuoussequential therapy, withdrawal bleeding was defined as that which occurred toward the end of or immediately after the administration of progestogen; bleeding that occurred at any other time was defined as breakthrough bleeding.

Results

Phase 1. All 12 patients completed the study. Their mean age was 51.8 years (range, 43 to 63 years); their mean height was 161 cm (range, 154 to 173 cm), and their mean weight 64.6 kg (range, 55 to 80 kg). Plasma levels of FSH and LH at the pretreatment visit were within the postmenopausal range for all 12 patients. However, subsequent analyses of the plasma values of estradiol and estrone showed that three patients were "perimenopausal" and the other nine were truly postmenopausal.11

The histologic findings obtained at the pretreatment biopsy and at the biopsy performed at the end of the third treatment month are presented in Table I. At the pretreatment assessment, no endometrium was obtained from six patients (50%). In four patients (33%), one of whom was perimenopausal, tissue was obtained but was so scant as to be unsuitable for assessment. One perimenopausal patient had nonsecretory endometrium (proliferative pattern but without mitotic figures), and the third perimenopausal patient had proliferative endometrium. After transdermal estradiol therapy, the most common finding was proliferative endometrium in seven patients (58%).

Two patients had no bleeding up to the time of the second biopsy; an additional two patients bled only at the commencement of therapy after the first biopsy. During therapy, withdrawal bleeding alone was reported by one patient; withdrawal bleeding together with breakthrough bleeding was reported by four patients, one of whom was perimenopausal; and breakthrough bleeding alone occurred in three patients, two of whom were perimenopausal. In five of the seven patients with breakthrough bleeding, the abnormal bleeding occurred just before or just after a treatmentfree week. The duration of withdrawal bleeding and also of breakthrough bleeding ranged from 1 to 7 days.

Phase 2. All eight patients completed the study. In the eighth patient, the second biopsy specimen was

Table I. Endometrial histologic findings before and during cyclical transdermal estradiol therapy

	No. of patients			
Endo netrial histology	Pretreatment	During therapy		
No tissue	6	1		
Scant material unsuitable for assessment	4*	2*		
Nonsecretory	1*	2		
Proliferative (Total 12	$\frac{-7\dagger}{12}$		

^{*}One perimenopausal patient.

Table II. Endometrial histologic findings during continuous-sequential estradiol/norethindrone therapy*

	No. of p	atients
Endome ^a rial histologès findings	During third month of cyclical therapy	During fifth and sixth months of continuous- sequential therapy
No tissue Scant material	l o	1
unsuitable for	2	1
Nonsecretory	1	1
Prolife-ative	$\frac{4}{8}$	<u>6</u>
Totai	8	8

^{*}The results from the third month of cyclical therapy are included for comparison.

taken in the fifth treatment month (exactly 4 weeks earlier than originally planned) to allow early analysis of data. Five of the patients were truly postmenopausal, and three were perimenopausal.11 Their mean age was 51.5 years (range, 44 to 64 years); their mean height was 160 cm (range, 156 to 165 cm), and their mean weight was 64.4 kg (range, 57 to 80 kg).

The histologic findings at the first and second visits are shown in Table II. Proliferative endometrium was the most common diagnosis on both occasions. There was no evidence of hyperplasia in any sample.

The number of days of bleeding each patient experienced per month is shown in Table III. The mean number of days of bleeding per month is expressed in two ways: as the mean value for the entire group and as the mean value for only those patients who experienced bleeding that month. The number (percentage) of patients with amenorrhea each month is also given.

Every patient experienced withdrawal bleeding at some stage during the study. One patient, who was postmenopausal, experienced breakthrough bleeding on three occasions in treatment months 3, 4, and 5. No

[†]Two perimenopausal patients.

Table III. No. of days of bleeding for individual patients during continuous-sequential estradiol/norethindrone therapy

	No. of days of bleeding, in month								
Patient group	. 1	2 .	3	4	. 5	6			
Individual patients (Patient No.)									
1	6 .	6	. 7	8	8	8			
2	5 '	4	4	4	4	0			
3	7	3	6*	7*	5*	5			
4	0	3	2	0	0	0			
5	0	0	0	. 0	0	3			
6	0	0	4	0	0	4			
7	9	11	7	10	4	12			
8	9	8	4	7 .	0 .	ND†			
All 8 patients (Mean ± SE)	4.5	4.4	4.3	4.5	2.6	4.6			
•	(1.4)	(1.4)	(0.9)	(1.4)	(1.1)	(1.6)			
Patients who bled (Mean ± SE)	7.2	5.8	4.9	7.2	5.3	6.4			
•	(0.8)	(1.3)	(0.7)	(1.0)	(1.0)	(1.6)			
Patients who experienced amenorrhea	3 -	2	1	3	4	` 1´			
(percentage)	(37.5%)	(25%)	(12.5%)	(57.5%)	(50%)	(14%)			

^{*}Breakthrough bleeding.

Table IV. Amount of bleeding for individual patients during continuous-sequential estradiol/norethindrone therapy

	Amount of bleeding,* in month									
Patient group	1 .	2	. 3	4	5	6				
Individual patients (Patient No.)										
1	2.8	2.5	2.4	2.9	2.9	2.9				
2	2.2	2.5	2.8	2.0	1.5	0				
3	3.1	1.3	2.2	2.3	1.6	1.2				
4	0	2.0	2.0	0	0	0				
5	0	0	0	0	0	1.0				
6	0	. 0	1.0	0	0 ·	1.3				
7	2.4	1.9	1.9	2.4	1.8	2.1				
, 8	1.1	. 2.1	1.0	1.0	0 .	ND†				
All patients (Mean ± SE)	1.5	1.5	1.7	1.3	1.0	1.2				
•	(0.5)	(0.4)	(0.3)	(0.4)	(0.4)	(0.4)				
Patients who bled (Mean ± SE)	2.3	2.1	1.9	2.1	1.9	1.7				
	(0.4)	(0.2)	(0.3)	(0.3)	(0.3)	(0.4)				

^{*}Scale: 0 = no bleeding; 1 = spotting; 2 = light bleeding; 3 = moderate bleeding; 4 = heavy bleeding. †No data gathered.

consistent changes were observed with time for the mean number of days of bleeding each month. Additionally, no consistent changes were observed with time in the number of patients who experienced amenorrhea each month. However, there were marked interpatient and intrapatient variations in the number of days of bleeding. For example, patient No. 7 bled every month, for 4 to 12 days. In contrast, patient No. 5 bled only once, for 3 days, after administration of noreth-indrone in the sixth month.

The mean amount of bleeding each month, as assessed by the patient, is shown in Table IV. The mean amount of bleeding is expressed in two ways: as the mean for the entire group and as the mean for only those patients who experienced bleeding that month.

No consistent changes were observed with time for the amount of bleeding, regardless of the way the mean value was expressed. Wide interpatient variations were observed each month (range of scores, 1.0 to 2.9), representing spotting to moderate bleeding. No patient consistently reported heavy bleeding.

Comment

In terms of endometrial proliferation and long-term safety, transdermal estradiol (Estraderm, CIBA Pharmaceutical Co., Summit, New Jersey) might be considered to possess certain potential advantages over oral preparations. The total dose of estradiol administered each day is low, only 0.05 mg, and conversion to estrone and estrone sulfate is much lower than with oral estro-

[†]No data gathered.

gens.6 For reasons stated in the introduction to this article, these characteristics might be expected to produce minimal endometrial proliferation.

In our first study (Phase 1), we observed that transdermal estradiol exerted a proliferative effect upon the endometrium in most postmenopausal women. After 3 months of cyclical transdermal estradiol, proliferative or nonsecretory endometrium was diagnosed in seven of the nine postmenopausal patients (78%) who at pretreatment had yielded either no endometrial tissue or tissue too scant for reliable assessment. The responses in the three perimenopausal women were less clearly defined. Tissue unsuitable for assessment was obtained from one patient both before and at the end of therapy; nonsecretory endometrium was transformed to a proliferative pattern by treatment in the second woman; and proliferative endometrium was diagnosed at both biopsies in the third patient.

Although cyclical transdermal estradiol induced endometrial proliferation in most patients, the extent of the proliferation appeared to be less than with oral therapies that we previously evaluated. 14, 15 In this study, we had planned to perform biochemical measurements (receptor content, DNA synthesis) as well as histologic assessments upon tissue obtained after transdermal administration of estradiol. However, in only three of the seven samples that showed proliferative changes after cyclical treatment was tissue obtained in quantities sufficient for biochemical analyses. Obviously, the data from those samples were too meager for meaningful conclusions to be drawn and therefore have not been included in this report. It has been our experience that, with oral therapies, tissue is usually obtained in quantities sufficient for biochemical analyses.14, 15 We stress that our comment that transdermal estradiol appears to stimulate the production of less endometrial tissue than do the oral therapies is a subjective assessment, since we did not weigh the tissue obtained at curettage.

The stimulation of less tissue with transdermal estradiol may be due to the lower plasma values of estradiol observed with this form of therapy as compared to those with the oral route of administration. With transdermal estradiol, the plasma levels of estradiol are approximately 50 pg/ml11; with oral estrogen preparations, they are approximately 140 pg/ml at steady state.5 Within the postmenopausal endometrium, estradiol is the predominant intranuclear estrogen, and the intranuclear levels correlate well with estradiol receptor content,16 which is an index of estrogenic stimulation.

The bleeding patterns during cyclical therapy also indicate endometrial stimulation. Ten patients experienced vaginal bleeding during therapy. In two patients, this occurred only at the commencement of therapy, immediately after the first biopsy, and may have resulted from this procedure. In the other eight patients, the bleeding occurred later during treatment and did not appear to be related to the biopsy. We regard the bleeding in these eight patients as having resulted from endometrial stimulation. It is important that there appeared to be no regular pattern to the bleeding. In the postmenopausal subjects, withdrawal bleeding alone was reported by only one patient, withdrawal pleeding and breakthrough bleeding occurred in three women, and breakthrough bleeding alone was observed n one patient. All three perimenopausal women had breakthrough bleeding, and one also experienced withdrawal bleeding.

We previously reported that the addition of a progestogen to oral estrogen therapy provides better control of the cycles and a more regular bleeding pattern.1,2 In addition, 12 days of progestogen therapy each month appears to protect against the development of endcmetrial hyperplasia that results from the use of oral estrogen.2.17 When progestogens are added, we have prescribed the estrogen therapy continuously, for 365 dars each year, to prevent the recurrence of symptoms in the treatment-free week. In previous studies with cyclical transdermal estradiol, we reported a trend for flusnes to recur in the treatment-free interval," and, therefore, in Phase 2 of these studies, we prescribed transdermal estradiol continuously, with 12 days of norethindrone added for each month.

Afte- 5 to 6 months of such continuous-sequential therapy in the present study, endometrium unsuitable for assessment was obtained from only one (13%) of eight patients; all other biopsy samples showed evidence of estrogenic stimulation. No cystic or atypical endometrial hyperplasia was observed in any biopsy specimen. During continuous-sequential therapy, all patients experienced vaginal bleeding at some time, but there was no consistent trend with time either in the frequency or duration of bleeding (Table III) or in the amount of bleeding. No patient consistently reported heavy bleeding (Table IV). The number of patients who experienced amenorrhoea did not change with time (Table III). Only one patient experienced breakthrough bleeding. She was truly postmenopausal, and there was no obvious explanation as to the cause of the

In conclusion, cyclical transdermal estradiol therapy induces endometrial proliferation in most patients, but to a lesser degree than observed with oral therapies, and does not appear to produce a regular pattern of bleeding. The addition of a low-dose progestogen produces a more acceptable bleeding pattern and, in this preliminary study, was not associated with the development of endometrial hyperplasia.

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Efficacy, acceptability, and metabolic effects of transdermal estradiol in the management of postmenopausal women

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Systemic side effects that result from oral administration of estrogens to postmenopausal women may be minimized by use of the transdermal route. We administered transdermal estradiol, 0.05 mg/day, cyclically for 3 months to 12 postmenopausal and perimenopausal women to study the efficacy, acceptability, and metabolic effects of this dosage form. The results showed that transdermal estradiol significantly increased plasma levels of estradiol and estrone and urinary concentrations of estradicl conjugates, and produced significant improvement in menopausal symptoms and vaginal cytologic findings. The patches were well tolerated and no systemic side effects were reported. No clinically significant adverse biochemical changes were observed. Plasma renin substrate and renin activity were unchanged during therapy. (AM J OBSTET GYNECOL 1985;152:1085-91.)

Key words: Menopausal syndrome, transdermal estradiol, efficacy, side effects

Estradiol is the predominant estrogen secreted by the ovary during the reproductive era. The initial decline in ovarian function during the perimenopause ultimately leads to an almost complete cessation of ovarian production of estrogens in the postmenopausal years. Thus, postmenopausal women are deficient principally in estradiol. It is well recognized that in some women this deficiency rapidly results in the development of vasomotor symptoms and in atrophic changes within the genital tract. Additionally, certain psychological symptoms, such as anxiety, forgetfulness, and difficulty in concentrating—collectively termed the "menopausal syndrome"-increase in frequency during the climacteric and early postmenopausal years.1 Ovarian failure also has long-term consequences, because postmenopausal women are at an increased risk of developing osteoporosis and certain fractures. Fortunately, replacement of estrogen during the climacteric and postmenopausal period not only relieves all the acute symptoms2 but also appears to protect against both the development of osteoporosis3 and the occurrence of related fractures.4.5

Currently, exogenous estrogen therapy is mainly prescribed by the oral route of administration. This has certain disadvantages. High doses of estrogen must be administered because of the rapid metabolism and inactivation of estrogens within the gut wall and liver. It has been estimated that up to 30% of the administered dose is inactivated even before it reaches the systemic

strated to control menopausal symptoms effectively without inducing adverse metabolic changes, then it will offer an attractive alternative to oral estrogen therapy. In this study, we examined the efficacy, acceptability, and metabolic effects of transdermal estradiol given to

circulation.6 Additionally, unwanted metabolic changes

may occur from enzyme induction within hepatic tissue,

and orally administered estrogens have been shown to

increase the levels of antithrombin III and renin substrate, ^{© 7} thus potentially increasing the risk of throm-

bosis and hypertension in predisposed individuals. Fur-

thermcre, after oral administration, only transient increases in the plasma levels of estradiol have been

observed, whereas there is a disproportionate rise in

plasma estrone that produces a nonphysiologic plasma

Pharmacokinetic studies have shown that estradiol

delivered via a transdermal system (Estraderm TTS,

CIBA-GEIGY Ltd., Basel, Switzerland) undergoes only

minimal metabolism, and the estradiol/estrone ratio

more closely approximates that seen during the repro-

ductive era.8 The potential advantage of delivering es-

tradiol transdermally is that gut wall and hepatic me-

tabolism may be avoided, thus not only allowing the

use of lower total daily doses but also diminishing the

risks of hepatic enzyme induction and its sequelae.

Therefore, if transdermal estradiol can be demon-

Patients and methods

postme lopausal women.

profile

This rial was an open trial conducted at King's College Hospital, London. Twelve symptomatic volunteers who were attending the Menopause Clinic were recruited for the study and gave informed consent. All

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Pretreatment During transdermal estradiol Cycle 3 Cycle 1 Cycle 2 Assessment Visit I Vist 2 Visit 3 Visit 4 Visit 5 Visit 6 Plasma FSH/LH/ estradiol/estrone Urinary estradiol conjugates Vaginal smears Blood pressure/weight Graphic Rating Scale booklets Biochemistry (SMAC*) Renin substrate/activity

Table I. Assessments and study visits during open trial of transdermal estradiol

Side effects

subjects were at least 6 months postmenopausal, with a plasma follicle-stimulating hormone level within the postmenopausal range. None had received estrogens in the 3 months prior to admission to the study.

The study lasted 4 months and involved six visits to the Clinic (Table I). The two pretreatment visits (visits 1 and 2) occurred 2 weeks apart, and therapy was started immediately after visit 2. The transdermal estradiol patches were prescribed for 3 weeks, followed by 1 treatment-free week; this sequence was repeated three times. Visits during the treatment phase occurred at the end of the third treatment week in each sequence, for a total of three visits (visits 3, 4, and 5). The final visit (visit 6) took place 2 weeks after visit 5, after discontinuation of therapy, and was a posttreatment assessment.

Each patch was designed to deliver 0.05 mg cf estradiol per day, and each patch was applied for 3 days. Patients were advised not to stick the patches near their breasts or genitalia but to use sites such as the abdomen, buttocks, or thighs. The patches were applied after bathing and drying. Once the patch was in situ it was not removed under any circumstances until completion of the 3-day period of wearing. (Patients were instructed to bathe and shower with the patch in place.) The number of patches that lost adhesiveness and became detached prematurely was recorded.

Throughout the study, we assessed the endocrine, physical, symptomatic, psychological, and biochemical status of each patient. Side effects attributable to transdermal estradiol were also recorded. The methodology used for these assessments is stated below; the timing of these assessments is shown in Table I.

Assessments

Endocrine. Plasma levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradioL and estrone, and urinary levels of estradiol conjugates were

determined by CIBA-GEIGY, Basel, Switzerland. FSH and LH were measured with reagents supplied by Diagnostic Products Corporation, Los Angeles, California. The sensitivities for FSH and LH were 2 and 3 U/L, respectively. Plasma estradiol was measured with reagents supplied by Radio Isotopen Service, Eidg. Institut fur Reaktorforschung, Wurenlingen, Switzerland; assay sensitivity was 2 pg/ml. Coefficients of variation between-assay were 12% to 15% and within-assay were 10% to 12%. Plasma estrone was measured with reagents supplied by Buhlman Laboratories, Basle, Switzerland. Sensitivity was 5 pg/ml. Coefficients of variation between-assay were 12% to 15% and withinassay were 10% to 12%. Urinary estradiol conjugates were determined after enzymatic hydrolysis (mixed glucuronidase-sulfatase from Helix pomatia, Sigma). Assay sensitivity was 80 pg/ml of urine. Coefficients of variation between-assay were 15% and within-assay were 10%.

Smears taken from the lateral vaginal fornices were immediately fixed in alcohol and analyzed by determination of the karyopyknotic index and also the maturation index.

Physical. Blood pressure was recorded with a conventional Accoson sphygmomanometer. Weight was measured on a set of Marsden's clinic scales.

Symptomatic and psychological. The efficacy of transdermal estradiol in relieving menopausal symptoms was assessed with the Graphic Rating Scale booklets, which were completed every week. The Graphic Rating Scale¹⁰ is a sensitive method of measuring physical and emotional changes and has gained widespread acceptance. It is designed around a line of fixed length for each symptom and emotional factor being studied; by marking the line, the patient uses it as a scale in a selfassessment of his or her present state. We previously reported that the Graphic Rating Scale is a sensitive method of measuring symptomatic and psychological

^{*}Serum multiple analyzer and computer.

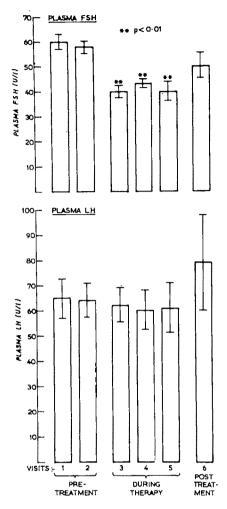


Fig. 1. Mean (±SE) plasma FSH and LH values before, during, and after transdermal estradiol. Significance of difference in comparison to pretreatment values. = = p < 0.01.

changes in climacteric and early postmenopausal women.2

In the present study, each Graphic Rating Scale booklet contained 35 questions. Only a few, however, were related to symptoms of the menopausal syndrome, such as hot flushes, sleep disturbance due to night sweats, vaginal dryness, irritability, anxiety, and difficulty in concentrating; the rest of the questions were included to disguise our interest in the truly menopausal symptoms, and some specifically sought information on estrogenic side effects, such as breast discomfort. For the assessment of hot flushes, patients were asked at weekly intervals to record the average daily number of hot flushes experienced during the previous week.

Biochemistry. A SMAC (serum multiple analyzer and computer) AutoAnalyzer (Technicon Instrument Co., San Francisco, California) was used for determining the plasma levels of creatinine, urate, urea and electrolytes, total protein, albumin, bilirubin, aspartate transami-

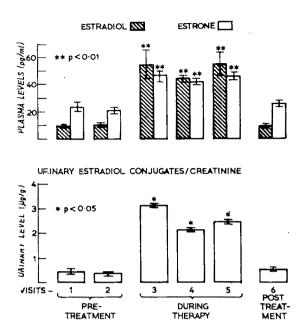


Fig. 2. Mean (±SE) plasma estradiol and estrone values and mean (±SE) urinary levels of estradiol conjugates before, during, and after transdermal estradiol. Significance of difference in comparison to pretreatment values. * = p < 0.05, ** =

nase, y-glutamyl transaminase, and inorganic phosphate. Flasma renin substrate was measured by a procedure based on methods previously described by Krakoff¹ and Waite et al. 12 The samples of plasma were diluted (20-fold) and incubated with purified renin (National Biological Standards Board, Holly Hill, London) under conditions previously shown to fully convert remin substrate to angiotensin I. The generated angioter.sin I was then measured by a specific radioimmuncassay. The results were expressed as nanograms of angiotensin I equivalents per milliliter of plasma. Renin activity was measured by radioimmunoassay as described by Roulston and MacGregor.13

Eide ef ects. Any adverse reaction that was potentially a s de effect of therapy was recorded by the patients throughout the study. The investigator looked for local reactions to the estradiol patches at visits 3, 4, and 5.

Statistical evaluations. The Student's t test was used for statistica. analyses. Probability values represent twotailed analysis.

Fesults

All 12 patients completed the study. Their mean age was 51.8 years (range, 43 to 63 years). Their mean height was 161 cm (range, 154 to 173 cm), and their mean weight was 64.6 kg (range, 55 to 80 kg).

Pasma levels of FSH and LH at the pretreatment visits were within the postmenopausal range for all 12 patients. However, subsequent analyses of the plasma

Measurement	Pretre	atment	Duri	After		
	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	treatment Visit 6
Systolic blood pressure (mm Hg)	120.6 ± 3.9	117.5 ± 3.5	113.0 ± 3.8	110.0 ± 3.4	119.0 ± 4.3	119.0 ± 4.7
Diastolic blood pressure (mm Hg)	71.3 ± 2.8	71.3 ± 5.1	$67.7* \pm 2.2$	68.7 ± 2.3	73.0 ± 2.3	73.0 ± 3.1
Weight (kg)	64.6 ± 2.4	64.2 ± 2.6	64.9 ± 2.4	65.0 ± 2.6	65.3 ± 2.6	65.6 ± 2.7

Table II. Mean (\pm SE) systolic and diastolic blood pressures and mean (\pm SE) weight before, during, and after transdermal estradiol

Significance of difference in comparison to pretreatment value.

estradiol and estrone values showed that for only nine patients were plasma estradiol values within the postmenopausal range, and only in these patients also were plasma levels of estrone equal to or higher than those of estradiol. We regard these nine patients as being truly "postmenopausal." In three patients, despite the presence of typical symptoms of estrogen deficiency and elevated concentrations of gonadotropins, the plasma levels of estradiol exceeded those of estrone. In our opinion, these elevated values of estradiol indicate diminished but not absent ovarian activity, and we regard these three patients as being "perimenopausal."

Obviously, since the presence of minimal ovarian activity is likely to influence certain results, all data were analyzed in three ways: for the 12 patients as a group, for the nine postmenopausal patients, and for the three perimenopausal patients. The perimenopausal data have been excluded if they were significantly different from the data for the postmenopausal patients.

Endocrine assessments. For the nine postmeno-pausal patients, plasma FSH and LH values declined during therapy and rose within 2 weeks of discontinuation of treatment (Fig. 1). Only the fall in plasma FSH values reached significance. Plasma concentrations of estradiol rose significantly (p < 0.01) during therapy; plasma levels of estrone also increased significantly (p < 0.01), but the relative rise was less (Fig. 2). The mean plasma value for estradiol during the 9 weeks of treatment was 51 pg/ml, and that for estrone was 45 pg/ml. A significant fourfold to sixfold increase in urinary estradiol conjugates (p < 0.05) was observed, reflecting the rise in plasma estradiol (Fig. 2).

Vaginal smears were taken in the pretreatment phase and in the final week of therapy in the third treatment cycle. On both occasions, eight smears were suitable for assessment. The karyopyknotic index significantly increased from a mean pretreatment value of 5.5 to 21.0 with therapy (p < 0.05). The maturation index results showed that the mean percentage of basal cells fell from 40 to 0, the mean percentage of intermediate cells rose

from 54 to 74, and the mean percentage of superficial cells rose from 6 to 26. Only the change in superficial cells reached significance (p < 0.05).

Physical evaluations. The mean (\pm SE) values for weight and systolic and diastolic blood pressures for all 12 patients are shown in Table II. No significant differences were observed for weight between any of the six visits. On two occasions (visits 3 and 4), mean systolic blood pressure was significantly lower (p < 0.05) than at pretreatment. Mean diastolic blood pressure was significantly lower at visit 3 than at pretreatment (p < 0.05).

Symptomatic and psychological effects. The changes in the average daily number of hot flushes experienced during the previous week are illustrated in Fig. 3. The results are expressed as a percentage of the mean of the two pretreatment values. There were no gross differences between results for the perimenopausal and the postmenopausal patients. The data shown are for the three perimenopausal patients and all postmenopausal patients who had hot flushes as a presenting complaint (seven of the nine patients).

With each successive treatment cycle there was a trend toward a further reduction in the number of hot flushes. For example, the pretreatment value for the average number of flushes experienced per day was 8.2; this fell to 2.3 in the third week of treatment cycle 1, was further reduced to 1.0 at the end of the second month of therapy, and fell to 0.5 in the final week of therapy. At the end of the study, the average number of hot flushes per day had declined from pretreatment levels by 91%. Additionally, there was a trend for fewer flushes to be experienced in treatment week 3 of each cycle, as compared to the first week, and for the flushes to increase in number during the treatment-free weeks (Fig. 3).

Fig. 4 shows the changes in the mean Graphic Rating Scale scores for menopausal symptoms from pretreatment to treatment with transdermal estradiol for all 12 patients. The scores shown are for the last week of

^{*}p < 0.05.

therapy in each treatment cycle. Transdermal estradiol exerted a significant beneficial effect on hot flushes, sleep disturbance, irritability, and poor concentration; the relief of vaginal dryness and anxiety just failed to reach significance (Fig. 4). The Graphic Rating Scale data for the nine postmenopausal patients alone showed a similar trend, but the beneficial effects on hot flushes and irritability were significantly greater, and the relief of anxiety reached significance (p < 0.05).

Transdermal estradiol also exerted significant beneficial effects on poor memory in the nine postmenopausal patients (p < 0.05) and on increased optimism in all 12 patients (p < 0.05) (data not shown).

Biochemistry. Biochemical data for all 12 patients are presented in Table III. The mean (±SE) value in treatment week 3 of the last cycle has been compared to the mean (±SE) value for the pretreatment levels. Plasma inorganic phosphate, albumin, and total bilirubin were significantly reduced (p < 0.05), but all individual values remained within the normal ranges. No other significant changes were observed.

Mean plasma renin substrate was 824 ng/ml (SE: ± 21.6) at pretreatment and was 729 ng/ml (SE: ± 36.2) in the third week of therapy in the final treatment month. Mean plasma renin activity was 1.77 ng/ml of plasma per hour (SE: ± 0.5) at pretreatment and was 1.09 ng/ml of plasma per hour (SE: ± 0.23) in the final week of treatment. These differences were not significant.

Side effects. No adverse systemic effects were reported by any of the 12 patients. The results from questions in the Graphic Rating Scale booklets specifically pertaining to the presence of symptoms often associated with exogenous estrogen therapy showed no significant increase in nausea, vomiting, or breast discomfort.

A total of 252 patches was applied, and only two patients reported, on three occasions each, poor adhesion of a patch; one of these patches became detached prematurely and was lost. Only two patients reported slight skin irritation from one of the patches. One patient reported local irritation on the first two visits during therapy, but after the site of application was changed, this problem disappeared.

The investigator's assessment of the skin application sites on visits 3, 4, and 5 reported "hardly visible redness" at the site of the last application in seven of the 12 patients, on a total of ten occasions. No allergic reaction to the patches was observed. Six of the patients had a previous history of allergy, three of them of topical allergy.

Comment

Estraderm (CIBA Pharmaceutical Co., Summit, New Jersey) is designed to deliver estradiol by the transder-

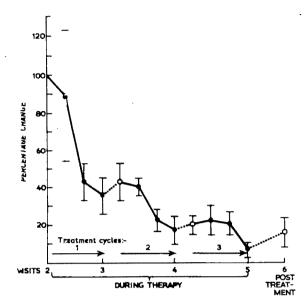


Fig. 3. Percentage change in mean (±SE) daily number of hot flushes. • — • = On treatment. 0 — 0 = Off treatment.

mal route in a continuous manner. We used the transdermal system that delivered 0.05 mg of estradiol daily. In our study, we observed a significant suppression of plasma FSH but not of LH. In terms of suppression of plasma FSH, our results agree with those of Laufer et al.8 and Powers et al.9 who also observed suppression of this hormone; in terms of LH, however, they differ from thos∈ of the above investigators, who did observe a significant suppression of plasma LH. Although Laufer et al.8 did not state the dose of estradiol used in their study, we think that they used skin patches that delivered approximately 0.1 mg of estradiol daily, and th's higher dosage may explain the discrepancy between our sets of results. This does not explain, however, the difference between our results and those of Pcwers et al.,9 who administered patches that delivered either 0.025, 0.05, or 0.1 mg daily and observed a dosedependent suppression of LH.

We observed plasma levels of estradiol between 44 and 55 pg/ml (mean, 51 pg/ml), which are within the range reported by Powers et al.9 They are also within the range previously reported by our group after oral administration of several other preparations: estradiol valerate (Progynova: Schering Chemicals), 1 and 2 mg daily; piperazine estrone sulfate (Ogen: Abbott Laborazories, North Chicago, Illinois), 1.5 and 3 mg daily; and conjugated equine estrogens (Premarin: Ayerst Laboratories, New York, New York), 0.625 and 1.25 mg daily. Unlike oral therapy, transdermal estradiol is not associated with a nonphysiologic rise in plasma estrone.6,14 We observed a mean estrone value of 45 pg/ m in this study; previously, we reported mean values between 115 and 328 pg/ml with the three oral estrogens referred to above.14

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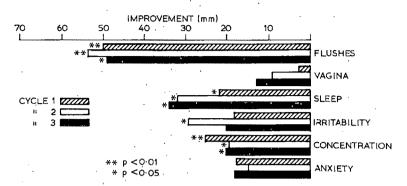


Fig. 4. Mean changes in Graphic Rating Scale scores (mm) in the last week of therapy in treatment cycles 1, 2, and 3, as compared to pretreatment. Significance of difference in comparison to pretreatment values. * = p < 0.05, ** = p < 0.01.

Table III. Mean (±SE) biochemical indices at pretreatment and at the final week of therapy in treatment cycle 3

Measurement .	Pretreatment			During treatment		
Creatinine (μM/L)	,	71.8 (±2.9)		73.6 (±3.7)		
Urate (mM/L)		$0.29~(\pm 0.01)$	•	$0.27 (\pm 0.01)$		
Urea (µM/L)		$5.10~(\pm 0.50)$	•	$4.55(\pm 0.40)$		
Sodium (mM/L)	,	$141.0 \ (\pm 0.80)$	-	$139.9 \ (\pm 1.1)$		
Potassium (mM/L)		4.1 (± 0.1)	. 9	$4.0 \ (\pm 0.1)$		
Bicarbonate (mM/L)		$25.0 \ (\pm 0.5)$		24.9 (± 0.7)		
Total protein (gm/L)	,	$68.2 \ (\pm 1.0)$		$68.6 \ (\pm 1.4)$		
Albumin (gm/L)		$44.4 \ (\pm 0.7)$		$42.9 \ (\pm 1.2)*$		
Total bilirubin (μM/L)		$8.9 \ (\pm 1.0)$		7.2 (± 0.9) *		
Aspartate transaminase (IU/L)	•	$21.3 \ (\pm 4.8)$		17.9 (± 2.1)		
Glutamyl transaminase (IU/L)		$16.8 \ (\pm 4.7)$		19.2 (± 6.1)		
Inorganic phosphate (mM/L)	. , .	$1.3 \ (\pm 0.04)$		$1.2 \ (\pm 0.05)^*$		

Significance of difference in comparison to pretreatment values.

The present study demonstrated the efficacy of this low dose of transdermal estradiol in the relief of menopausal symptoms. Significant beneficial effects were observed on the vaginal epithelium, as assessed by the karyopyknotic index and maturation index. We agree with Laufer et al.8 that vaginal cytologic findings are a sensitive marker of estradiol action. Relief of menopausal symptoms was assessed by the Graphic Rating Scale, which showed that transdermal estradiol exerted significant beneficial effects on hot flushes, sleep disturbance due to night sweats, poor concentration, irritability, and optimism. Anxiety and poor memory were significantly relieved in the postmenopausal patients. The present results compare favorably with those previously reported by our group with oral conjugated equine estrogens, 1.25 mg daily.2 We think that our present results represent a genuine response to therapy. We believe that it is unlikely that the results represent a placebo effect, since in a previous study, which was placebo controlled and in which identical assessments were used, these menopausal symptoms did not respond to placebo.2

We observed a decline in the average number of hot

flushes experienced each day. An important observation was that each successive treatment cycle produced further beneficial effects. This raises the possibility that one month of treatment may be too short a time for a reliable interpretation of results and may explain why Laufer et al., who evaluated results after only 3 weeks of therapy, observed suboptimum responses. In our study, the decrease in hot flushes observed at the end of the third month of therapy was a decline by 91% from the pretreatment value.

Mean plasma levels of inorganic phosphate, albumin, and total bilirubin were reduced (p < 0.05). The clinical significance of these changes is unclear but, in our opinion, is likely to be minimal because none of the individual values was outside the normal range. We confirm the findings of Laufer et al. that transdermal estradiol does not significantly alter plasma renin substrate. We also demonstrated no significant change in renin activity. This is in contrast to the significant increases in renin substrate observed with oral estrogen therapy and illustrates the lack of potentially adverse effects with the transdermal route of administration.

No significant increases in weight or systolic or dia-

^{*}p < 0.05.

stolic blood pressures were observed. Systemic side effects attributable to transdermal estradiol were not reported by any of the 12 patients, and specific questioning by means of the Graphic Rating Scale booklets showed that transdermal estradiol did not cause nausea, vomiting, or breast tenderness. Local irritation from the patches was infrequent and was reported on only four occasions out of a total of 252 applications. "Hardly visible redness" was observed at the site of the last application by the investigators on a total of only 10 occasions. No allergic reactions occurred, although three patients had a history of topical allergy. Adhesion was good. Only six patches became partially dislodged and only one was lost because of premature detachment.

In conclusion, a transdermal system that delivers 0.05 mg of estradiol daily appears to relieve menopausal symptoms effectively and does so without causing adverse metabolic or local changes.

We wish to thank Dr. L. Schenkel, Mrs. D. Barlier, and Mr. T. Gschwind, at CIBA-GEIGY, Basel, Switzerland, for performing the endocrine assays and for their help with the statistics; and Dr. G. A. MacGregor, Charing Cross Hospital, Hammersmith, London, for performing the plasma renin substrate and renin activity assays.

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A double-blind comparative study of Estraderm and Premarin in the amelioration of postmenopausal symptoms

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Patients whose postmenopausal symptoms were being satisfactorily controlled with conjugated equine estrogens, either 0.625 mg/day (n = 57) or 1.25 mg/day (n = 67), participated in a study that compared the efficacy of these oral regimens with that of 17β-estradiol, 0.1 mg/day, administered through intact skin. The study was a multicenter, double-blind, randomized, parallel-group trial during which two thirds of the patients who received each dosage of conjugated equine estrogens were changed to an estradiol transdermal system while the remainder continued with conjugated equine estrogens. A total of 124 patients was included in the analysis of efficacy. The analysis revealed no significant differences between the estradiol transdermal system and conjugated equine estrogens in control of hot flushes or other postmenopausal symptoms and no statistically significant differences between treatment groups in regard to estrogen-related side effects. Minor topical reactions to the transdermal systems were reported during only about 20% of study weeks. Thus, transdermal estradiol, 0.1 mg/day, appears to be equally effective as conjugated equine estrogens, 0.625 or 1.25 mg/day, for controlling postmenopausal symptoms and is well tolerated. (AM J OBSTET GYNECOL 1985;152:1092-9.)

Key words: Postmenopausal symptoms, oral estrogens, transdermal 17β -estradiol, double-blind study

Postmenopausal women who are receiving oral administration of either conjugated equine estrogens or 17β-estradiol generally have higher peak concentrations of estrogen in plasma than do premenopausal women and much lower estradiol/estrone ratios.¹ In addition, oral estrogen replacement therapy markedly elevates certain hepatic proteins.¹ These undesired effects of treatment arise principally from first-pass hepatic metabolism. In the liver, conjugated estrogens and estradiol are both substantially metabolized to estrone. Consequently, a dosage high enough to raise estradiol in plasma to therapeutic levels also disproportionately increases the concentration of estrone. The estradiol/estrone ratio is reduced further by the much shorter half-life of estradiol than that of its metabolite.

The aim in developing a dosage form for administering 17β-estradiol through intact skin was to avoid first-pass effects and to provide effective estrogen replacement therapy by restoring premenopausal estrogen balance. The system developed (Estraderm, estradiol transdermal system, CIBA Pharmaceutical Co., Summit, New Jersey) releases the natural ovarian hormone estradiol in a pattern that mimics its physiologic secretion—that is, continuously at low rates directly into the bloodstream.

From the ALZA Corporation and the CIBA-GEIGY Corporation. Reprint requests: Dr. Virgil A. Place, ALZA Corporation, 950 Page Mill Road, P.O. Box 10950, Palo Alto, CA 94303-0802.

The estradiol transdermal system is a thin, multilayered unit that contains a drug reservoir, a rate-controlling membrane, and an adhesive layer. The systems administer estradiol at controlled rates of 0.025, 0.05, or 0.1 mg/day in vivo as indicated for estrogen replacement therapy. Bioavailability data2 have shown that the systems produce levels of estradiol and estradiol/estrone ratios in postmenopausal women that mimic those observed premenopausally in the early follicular phase. In one study² concentrations of estradiol in plasma rose from about 7 pg/ml at pretreatment to approximately 25, 40, and 75 pg/ml during use of the transdermal system. Pretreatment levels of estrone were approximately 30 pg/ml and rose to 30 to 60 pg/ mg; the estradiol/estrone ratio rose from about 0.2 to approximately 1, within the premenopausal range. Treatment with conjugated equine estrogens, on the other hand, produced an estradiol/estrone ratio of 0.25, which is within the untreated postmenopausal range. Levels of hepatic proteins were not significantly elevated by the transdermal administration of estrogen.

In the study reported here, the efficacy of the transdermal systems that delivered 0.1 mg/day of estradiol was compared with that of conjugated equine estrogens (Premarin, Ayerst Laboratories, New York, New York), 0.625 and 1.25 mg/day. Inclusion criteria specified admission only of patients whose menopausal symptoms were under satisfactory control with these maintenance doses of conjugated equine estrogens.

Table I. Study design of Estraderm (estradiol transdermal system: versus Premarin (conjugated equine estrogens) for control of postmenopausal symptoms

Weeks 1-3	Week 4	Weeks 5-7	Week 8
Premarin, 0.625 mg/day (n = 57)	Placebo	Group 1—Premarin, C.625 mg/day	Placebo
		Group 2 (n = 41)—Estraderm,	Placebo
Premarin, 1.25 mg/day (n = 67)	Placebo	Group 3—Premarin, I.25 mg/day	Placebo
		Group 4 (n = 47)—Estraderm, (.1 mg/day	Placebo

Table II. Calculation from daily data of single treatment-period value for hot flushes

Daily data							Weekly	Treatment	
Week	1	2	3	4	5	6	7	valuė́	period value
1							_	Sum7	, .
2								Sum -	→ Average of
3							_	Sum	sums
4								Sum —	→ Sum
5								Sum7	
5								Sum —	→ Average of
7								Sum_	sums
R								Sum	→ Sum

Methods and material

Ten investigators participated in the study.*

Subjects. Those patients who were entered into the study were required to be in good health or to have any significant disease conditions adequately controlled. Of 166 patients admitted, 79 had been maintained on conjugated equine estrogens, 0.625 mg/day, and 87 on 1.25 mg/day. About 75% of those admitted and of those included in the analysis of efficacy (n = 124) had undergone oophorectomy (n = 93) at least 1 month previously. The remainder had not menstruated for at least 6 months. The mean age of patients included in the efficacy analysis was 47 years (range, 25 to 66 years); mean height was 65 inches (range, 57 to 71 inches); and mean weight was 146 pounds (range, 83 to 264 pounds). Months since last menses averaged 54 (range, 3 to 282 months), and months of use of conjugated equine estrogens averaged 10 (range, 3 to 24 months).

Study design. This was a double-blind, randomized, parallel-group trial. Throughout the trial, regardless

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Table III. Mear, number of hot flushes per week for Premarin and Estraderm treatment groups

	Mean No. of hot flushes			
Treatment group	Weeks 1-3	Weeks 5-7		
1 (0.625 mg/day of Premarin only)	5.58	5.27		
2 (0.625 mg/day of Premarin, then Estraderm, 0.1 mg/day)	7.15	4.23		
3 (1.25 mg/day of Premarin only)	4.03	2.98		
4 (1.25 mg/day of Premarin, then Estraderm, 0.1 mg/day)	4.30	5.16		

Differences not significant (p > 0.10, within-patient com-

of treatment, each patient wore two transdermal systems (each delivering either 0.05 mg of 17β-estradiol per day or no drug), which they changed twice weekly, and took one capsule daily (containing either one or two 0.625 mg tablets of conjugated equine estrogens or no drug).

After an evaluation had been made and informed consent obtained, patients continued their maintenance dosage of conjugated equine estrogens for 3 weeks while vearing placebo transdermal systems. They then entered a 1-week phase in which they received placebo doses of both oral and transdermal administrations. The following week they were randomly assigned to one of four treatment groups, in the ratio of 2 to 1 for transdermal estradiol to conjugated equine estrogens in each group (Table I).

The administration of placebo in weeks 4 and 8 was designed to mimic the cycling of estrogen administration frequently prescribed in clinical practice.

Recording of data. Patients received diaries (Fig. 1) with instructions to record daily the number and intensity (on a scale of 0 to 9) of hot flushes and the frequency or intensity of other menopausal symptoms. They also recorded possible estrogenic side effects, such as breast tenderness, spotting, or bleeding. Each week at their visit to the clinic, patients returned the diaries and any unused capsules or transdermal patches

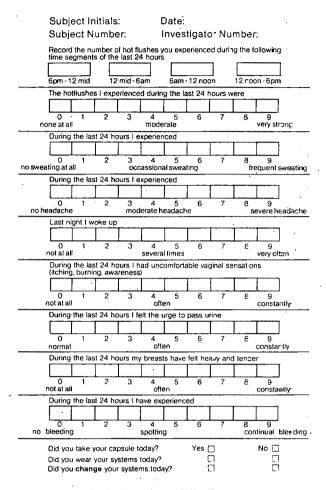


Fig. 1. Patient's daily diary.

and obtained new supplies. At enrollment and at the end of each week, patients were seen by the clinical research coordinator, who recorded continuing symptoms. At enrollment and at the end of weeks 3 and 7, subjects were seen by the investigator; global assessments were made at week 3 or 7 or at early termination.

Data evaluation and statistical analysis. Each patient's daily data on hot flushes were reduced to one value for each treatment period (Table II). The weekly value for hot flushes was the sum of the daily number of hot flushes; for severity, the weekly value was the sum of the daily scores divided by the number of days with flushes.

Statistical procedures included the signed rank (one-sample) Wilcoxon test for within-patient comparisons (e.g., first versus second period treatment outcomes); a two-sample Wilcoxon (Mann-Whitney) test for analysis of differences in outcomes between treatment groups; a two-sample t test for comparison of patients' attributes in the four groups; and Spearman's rank correlation for analysis of correlation within patients between outcomes from first and second treatment periods.

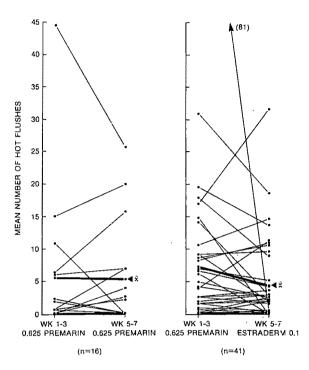


Fig. 2. Mean number of hot flushes in individual patients during weeks 1-3 and 5-7 in groups 1 and 2.

A power analysis (80% level) of the two-sample Wilcoxon test on hot flush outcome was conducted with standard methodology.

Results

With one exception, all data discussed in this section pertain to the 124 patients who completed at least 6 weeks of the study. (Systemic and topical side effects were analyzed for all 166 women admitted.) Of the 124 patients included in the analysis of efficacy, 118 completed 8 weeks of the study, five completed 7 weeks, and one completed 6 weeks.

Premature discontinuations. A total of 51 patients discontinued the study. Reasons for termination were grouped in five categories: side effects (17 patients), ineffective medication (five), poor compliance (nine), lost to follow-up (one), and "other" (19). Thirty-seven patients (73% of the 51) dropped out in the first 4 weeks and thus never received transdermal estradiol. A total of 6 patients discontinued the study while wearing estradiol transdermal systems, one for skin irritation and two for other reasons possibly related to the medication.

Of the total 13 discontinuations attributed to skin irritation, 12 occurred during the wearing of placebo transdermal systems (see *Topical side effects*). Other discontinuations attributed to side effects, as well as the five attributed to ineffective medication, occurred in patients who had been treated only with conjugated equine estrogens.

Table IV. Within-patient group treatment comparison: medians for number of hot flushes per week in Premarin and Estraderm treatment groups

	Dosage (14/2	
Weeks Premarin		Estraderm	n	Media⊒ hot flushes	Wilcoxon signed rank test p(two-sided)	
Patients in analy.	sis of efficacy ($n = 12$?4)				
1-3 5-7	0.625 0.625		16	0.7 1.2	0.62	
1-3 5-7	0.625	0.1	41 .	2.7 2.0	0.25	
1-3 5-7	1.25 1.25		20	1.0	0.16	
1-3 5-7	1.25	0.1	47	2.0 2.3	0.92	
Patients having i	undergone oophorecton	ny (n = 93)				
1-3 5-7	0.625 0.625		12	1.5 1.2	0.93	
1-3 5-7	0.625	0.1	24	3.8 2.8	0.34	
1-3 5-7	1.25 1.25		18	2.3 1.5	0.16	
1-3 5-7	1.25	0.1	39	2.0 2.3	0.98	

Table V. Between-subject comparison: mean number of hot flushes per week in Premarin and Estraderm treatment groups

Group			All patients analyz $(n = 124)$	ed	Patients who underwent oophorectomy $(n = 93)$			
Treatments (weeks 5-7)	Dosage (mg/day)	n	No. of het flushes			No. of hot flushes		
			Mean*	p#	n.	Mean ^{:*}	pt	
Premarin Estraderm	0.625 0.1	16 41	-0.31 -2.92	0.30	12 24	-0.81 -4.23	0.51	
Premarin Estraderm	1.25 0.1	20 47	-1.05 0.86	0.35	18 39	-1.22 0.83	0.37	

^{*}Average score for study weeks 5, 6, 7 minus average for weeks 1, 2, 3. Positive value = increase in symptoms relative to weeks 1, 2, 3.

Discontinuations for "other" reasons included 14 that were not related to medication and three that were possibly related to the study, such as depression. Other reasons were fibrocystic disease (one patient, week 4), and endometrial hyperplasia (one patient), diagnosed from a biopsy specimen taken on the day of enrollment.

Compliance. Missed medication events, assessed by examination of the diary, represented 1% or less of all prescribed medication or placebo administrations. Among the four groups, noncompliance never exceeded 1.4% for any placebo or treatment regimen.

Comparative efficacy in controlling hot flushes. Neither within- nor between-patient comparisons revealed any statistically significant differences in frequency of hot flushes between the estradiol transdermal system and conjugated equine estrogens.

Results of a within-patient comparison of the frequency of hot flushes in weeks 1 to 3 versus weeks 5

to 7 howed no statistically significant differences (p > 0.10) in the mean number of hot flushes per week for the fcur groups (Table III).

Figs 2 and 3 present the mean number of hot flushes in individual patients during weeks 1 to 3 and 5 to 7. The percentage of women within each treatment group that exhibited no flushes was consistently between 25% and 50%. Those percentages reflect the principal criterion for admission to the study, i.e., currently satisfactory treatment of hot flushes with conjugated equine estrogens, 0.625 or 1.25 mg/day.

Because the data on number of hot flushes are distinctly nonnormal, Table IV presents the medians for number of hot flushes per week for within-patient comparisons of treatment. The calculation was carried out for all 124 patients included in the analysis of efficacy and was also done separately for the 93 patients who had undergone oophorectomy. The latter were signif-

[†]Two-tailed probability for Wilcoxon two-sample test. (Significant difference if p < 0.10.)

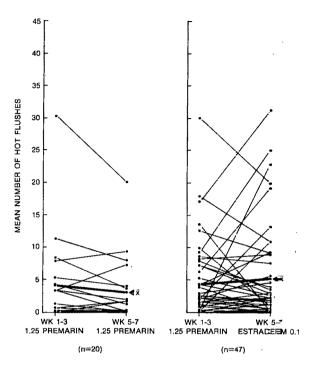


Fig. 3. Mean number of hot flushes in individual pæients during weeks 1-3 and 5-7 in groups 3 and 4.

icantly younger than patients with natural menopuse (mean age, 45 versus 52 years, p = 0.001) and had a shorter interval between their last menses and entry into the study (47 versus 75 months, p = 0.04).

Results of the statistical analysis using the one-sample Wilcoxon (signed rank) test showed that in no instance were the differences in the number of hot flushes significant (p > 0.10) over weeks I to 3 compared to weeks 5 to 7, either for the patients who had undergone oophorectomy or for the entire group. The patients who had undergone oophorectomy, however, had a consistently higher incidence of hot flushes that did those with natural menopause.

Approximately 75% of the women experienced eight or fewer hot flushes per week during the study. So verity, scored daily between 0 (mildest) and 9 (most severe), was low; mean values during treatment ranged between 0.83 and 1.69, all with similar standard errors of approximately 0.3.

In the between-subject comparison, the average score for mean number of hot flushes per week for weeks 1, 2, and 3 was subtracted from the average for weeks 5, 6, and 7. This eliminated the absolute difference in mean weekly occurrence of hot flushes among the four groups. Table V presents the results for all patients and for the subgroup of patients who underwent cochorectomy. Patients who had been using 0.625 mg/dzy of conjugated equine estrogens tended to have fewer hot flushes when receiving the estradiol transdermal sys-

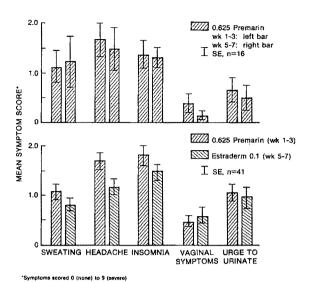


Fig. 4. Mean scores for postmenopausal symptoms recorded in the daily diaries for groups 1 and 2.

tem, and patients who had been taking 1.25 mg/day of conjugated equine estrogens tended to have more flushes with the transdermal system. In none of the comparisons, however, were the differences between the group using the estradiol transdermal system and the group taking conjugated equine estrogens significant (p > 0.10).

Results of the statistical power analysis showed that this trial, as conducted, had an 80% chance of rejecting the hypothesis of treatment equality if the treatments differed by 3.0 flushes per week for conjugated equine estrogens, 0.625 mg/day, versus transdermal estradiol, or by 1.9 flushes per week for conjugated equine estrogens, 1.25 mg/day, versus transdermal estradiol.

Efficacy in controlling other menopausal symptoms. Daily data for five menopausal symptoms (sweating, headache, insomnia, vaginal discomfort, urge to urinate) were reduced to a single treatment period value in the same manner as for severity of hot flushes. Figs. 4 and 5 present the mean scores and standard errors for these symptoms. All average scores were less than 2 on a scale of 0 to 9. Statistical analyses showed that the transdermal estradiol and conjugated equine estrogens groups did not differ significantly (p > 0.10) in regard to the severity of the five symptoms recorded.

Global assessments. At the end of weeks 3 and 7, the investigator rated each patient's condition as very much improved, much improved, minimally improved, unchanged, minimally worse, much worse, or very much worse. The week 3 comparison was made relative to the patient's status at enrollment, and the week 7 rating was relative to the patient's status during week 3. The global evaluations at week 3 were nearly the

Table VI. Global evaluations at week 3 and week 7.

	i				
Group	No charge	, Improved	Worse		
	Week 3				
1 (0.625 mg/day of Premarin only)	31	50	19		
2 (0.625 mg/day of Premarin, then Estraderm, 0.1 mg/day)	61	34	5		
3 (1.25 mg/day of Premarin only)	56	39	5		
4 (1.25 mg/day of Premarin, then Estraderm, 0.1 mg/day)	57	36	7		
		Week 7			
1 (0.625 mg/day of Premarin only)	50	19	31		
2 (0.625 mg/day of Premarin, then Estraderm, 0.1 mg/day)	49	32	19		
3 (1.25 mg/day of Premarin only)	56	22	22		
4 (1.25 mg/day) of Premarin, then Estraderm, 0.1 mg/day)	54	23	23		

Table VII. Reported bleeding by treatment group, in patients included in analysis of efficacy

Treatment group	Weeks of study								Total	4
	1	2	3	4	5	6	7 .	8	days of bleeding	Average score
	[*				2				1	2.0
I							2	112	4	1.5
				444	2			44	6	3.7
	,							1.	I	1.0
					4			994	4	6.5
2	₹1						2698	887531.	11	5.4
	11			3	2			4442	8	2.6
	4444	44	444	44	4			4574	16	4.2
								4333	4	3.2
				4	3				2	3.5
								23	2	2.5
								555	3	5.0
	¹ 111	1111				33 444 42		2321	18	2.2
4	{4			:			65966	6576554	13	5.7

^{*}The data for each day of the week (as reported by patients) are represented by either a dot (score of 0: no bleeding) or by a spotting (1-4) or bleeding (5-9) score. (See Fig. 1.)

same for treatment groups 2, 3, and 4. Group 1 (conjugated equine estrogens, 0.625 mg/day) showed a substantially lower percentage of patients with "no change," with the remainder divided about equally between "improved" and "worse" (Table VI).

Week 7 global evaluations of all groups were similar in percentage of patients unchanged, and groups 3 and 4 were almost identical in all categories.

Side effects

Estrogen-related side effects. Mean scores for spotting or bleeding did not differ among the treatment groups. A total of 18 of the 124 patients included in the analysis reported spotting or bleeding, of whom four had undergone oophorectomy and hysterectomy. These four had spotting only, which probably originated from the vagina.

In 10 of the 14 patients with intact uteri, spotting or

bleeding occurred after estrogen withdrawal (Table VII) and sometimes carried over into the first day of resumption of estrogen replacement therapy. Most spotting scores (74%) were in the range of 1 to 4. Four patients had spotting or bleeding at times other than off-trealment weeks. Two had bleeding scores as high as 9 in week 7 and continuing into week 8. In both, a diagnosis of mild endometrial hyperplasia had been made, and both had been treated with medroxyprogesterome acetate prior to enrollment. A third patient may not have been completely menopausal, since her last period had occurred 10 months prior to enrollment. The fourth patient had had a biopsy on the day of enrolment.

A total of 70 patients reported breast tenderness at some time during the study. Means of the scores were low, with a range from 0.69 to 1.65 on a scale of 0 to

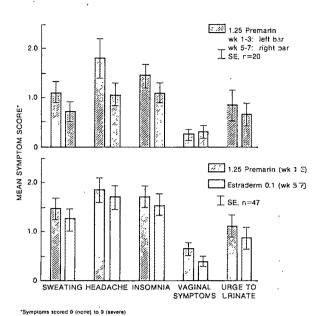


Fig. 5. Mean scores for postmenopausal symptoms recor=d in the daily diaries for groups 3 and 4.

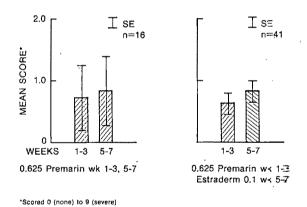


Fig. 6. Mean scores for breast tenderness recorded in the delay

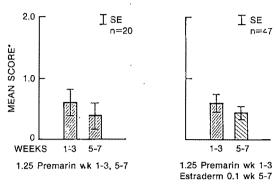
diaries for groups 1 and 2.

9, and were similar for the different groups over the weeks of treatment (Figs. 6 and 7).

Systemic side effects. Systemic side effects reported were similar in nature and incidence among all regimens, including placebo. Overall incidence was less than 10%, based on weeks of exposure. The number of events reported was about equally distributed during the weeks of the different treatments.

Among the 166 patients who enrolled in the stuzz, 38 reported a total of 73 symptoms, such as fatigate, moodiness/depression, bloating, and nausea. Investigators recorded only two of these as definitely being related to medication (mild abdominal bloating and moderate irritability, both of which occurred during transdermal estradiol treatment).

Topical side effects. Since terminology for describing skin reactions was not controlled, patients and inces-



*Scored 0 (none) to 9 (severe)

Fig. 7. Mean scores for breast tenderness recorded in the daily diaries for groups 3 and 4.

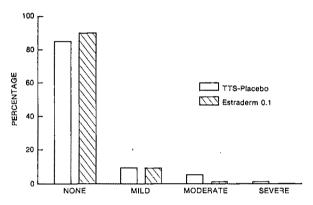


Fig. 8. Reported incidence of skin irritation (percentage of patient weeks). TTS, Transdermal therapeutic system.

tigators used various descriptions, such as redness, rash, itching, dermatitis, and swelling. On the basis of these reports, reactions were characterized as mild, moderate, or severe.

Overall, the incidence of skin irritation with the transdermal systems, both estradiol and placebo, was low. No topical reactions were reported during more than 80% of study weeks (Fig. 8).

The incidences of mild, moderate, and severe reactions were roughly equivalent for the four treatment groups. All but nine reactions reported were mild or moderate, and all reactions were transitory. The predominant reaction reported was erythema, described as a transient redness or rash. Among the 13 patients whose early terminations from the study were attributed to skin irritation, six had severe effects (severe rash, redness, or itching). All of these occurred during wearing of the transdermal placebo systems, as did the milder reactions in the seven other patients who discontinued treatment early.

All skin reactions were restricted to the general area in which the system was applied. None was the result of flare-up on a previous application site.

In conclusion, Estraderm (estradiol transdermal sys-

tem), 0.1 mg/day, was as effective as Premarin (conjugated equine estrogens), 0.625 or 1.25 mg/day, in controlling hot flushes and other postmenopausal symptoms in patients who had previously obtained satisfactory relief with Premarin. The estradiol transdermal system was well tolerated, as evidenced by the low incidence of estrogen-related, systemic, and topical side effects. The incidences of systemic side effects were similar among the transdermal estradiol, conjugated equine estrogens, and placebo groups, and the incidences of estrogen-related effects (breast tenderness, bleeding) were not significantly different between patients treated with transdermal estradiol and those

treated with conjugated equine estrogens. Most of the skin reactions reported were mild erythema, and all reactions were transient. They occurred with approximately equal incidences in the estradiol and placebo treatment groups.

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Pharmacokinetics and pharmacodynamics of transdermal dosage forms of 17β-estradiol: Comparison with conventional oral estrogens used for hormone replacement

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This open-label, multiple-crossover study compared the pharmacokinetics and pharmacodynamics of transdermal 173-estradiol and two oral forms of estrogen replacement therapy in postmenopausal women. The transdermal systems delivered either 0.025, 0.05, or 0.1 mg/day; oral dosages were 2 mg of micronized 17β-estradiol or 1.25 mg of conjugated equine estrogens. Transdermal estradiol provided serum and urinary levels of estradiol conjugates typical of the early follicular phase of the premenopausal woman and an estradiol/estrone ratio that approximated 1. The increments of both serum and urinary estradiol showed dose proportionality. Serum levels of estradiol obtained 24 hours after oral administration of estrogens were in a range similar to the steady-state levels obtained with transdermal estradiol délivery. Oral estrogens, however, induced an excessive rise in estrone to levels far beyond those observed in premenopausal women. Continuous application of transdermal estradiol over 3 weeks did not result in any accumulation of estradiol or estradiol conjugates. After only three doses of oral estrogens, there were signs of retention of estrogens. Suppression of gonadotropins by oral and transdermal administration of estrogens was in a similar range. This observation supports the conclusions that levels of circulating estradiol are relevant to efficacy, and that excessively high levels of estrone after oral administration of estrogens merely represents a nonphysiologic precursor or metabolite pattern. (AM J OBSTET GYNECOL 1985;152:1099-106.)

Key words: Transdermal estradiol, pharmacokinetics, pharmacodynamics, oral estrogen, postmenopausal therapy

Oral estrogen replacement therapy can relieve postmenopausal vasomotor symptoms, minimize atrophic changes in the vagina, and prevent osteoporosis, but

From the ALZA Corporation and the CIBA-GEIGY Corporation. Reprint requests: Marilou Powers, ALZA Corporation, 950 Page Mill Road, P.O. Box 10950, Palo Alto, CA 94303-0802. the use of it is associated with undesired effects that appear related to dose and duration of treatment.

The oral estrogens most commonly used are conjugated equine estrogens (Premarin, Ayerst, New York, New York) and a micronized form of 17β-estradiol (Estrace, Mead Johnson, Evansville, Indiana), the administration of which provides premenopausal levels of es-

Table I. Subject characteristics (n = 14)

	Age (yr)	Height (inches)	Weight (pounds)	Scrum estradiol (pg/ml)	Serum estrone (pg/ml)
Mean	62.6	63.1	132.3	4.1	33.3
SE	1.5	0.6	3.5	0.4	3.8
Range	52-73	58-67	105-149	3-8	21-52

tradiol but gives rise to nonphysiologic levels of -s.rone and its conjugates because of the substantial in estinal and hepatic metabolism of these estrogens. Moreover, oral estrogens cause elevation of hepatic proteirs, such as renin substrate.¹

Since parenteral administration of estradiol arcids first-pass hepatic metabolism, it should prevent some of these undesired effects, as well as reduce the dose required. For this reason, a transdermal ther peutic system (Estraderm, CIBA-GEIGY, Summit, Now Jersey) has been developed for controlled delivery of 17β-estradiol through intact skin to raise serum levels of estradiol into the physiologic premenopausal range. The studies described here compared the pharmacokinetics and pharmacodynamics of estradiol transcermal systems and two oral forms of estrogen replacement therapy in postmenopausal women.

Methods and material

Subjects. The study population (Table I) irreladed 14 postmenopausal women who provided in ormed consent and were in good health, as shown by medical history, physical examination, blood chemistry profile, and urinalysis. Subjects who previously used e trogen discontinued it 30 to 90 days before entering the study.

Study design and medications. This was ar epenlabel, multiple-crossover study. Each subject recived three treatments (micronized 176-estradiol, transdermal estradiol at three dosage levels, and conjugated equine estrogens), as shown in Table II. The study included a delivery-rate comparison phase for the three dosage levels of the estradiol transdermal syst m and a continuous-wearing phase for the system the delivered 0.05 mg/day.

Estraderm systems range in area from 5 cm² 1c 20 cm². Each consists of a backing membrane, drug reservoir, rate-controlling membrane, adhesive layer, and protective liner that is removed before the allesive layer is applied to the skin. Skin application sies and system adhesion were monitored while the system was used. Pharmacologic responses were monitored throughout the study.

Laboratory studies. Samples of serum were callected for measurement of estradiol, estrone, follicle-stimulating hormone (FSH), and luteinizing hormone (LH). Early morning samples of urine were collected caily for determination of urinary estradiol and estrone con-

jugates and creatinine. During continuous wearing of the estradiol transdermal system, serum levels of sex hormone binding globulin were also measured.

Assays for serum levels of estradiol, estrone FSH, LH, and sex hormone binding globulin were conducted at Endocrine Sciences, Tarzana, California. Estradiol and estrone were measured by a modification of radioimmunoassay procedures described by Wu and Lundy.³ FSH and LH were assayed by radioimmunoassay methods developed at Endocrine Sciences; results were expressed in international units (mIU/ml), as defined by the World Health Organization (FSH standard 69/104, LH standard 68/40). Sex hormone binding globulin was measured directly in serum by means of a displacement technique developed by Nugent et al. (unpublished).

Urinary estradiol and estrone conjugates were determined by enzymatic hydrolysis/extraction followed by a specific estradiol or estrone radioimmunoassay method. Urine creatinine was measured by the Jaffe reaction with the AutoAnalyzer (Technicon Instrument Co., San Francisco, California). All urine assays were conducted at CIBA-GEIGY, Ltd., in Basel, Switzerland.

Statistical analysis. Linear regression was used for analysis of the relationship between estradiol transdermal delivery rates and serum and urine levels of estradiol. The paired t test with two-tailed probabilities was used for analysis of the significance of mean differences in outcome variables for pairs of treatment conditions undergone by the subject group. The Dunnett t test (two-tailed) for multiple comparisons with a control was used for evaluation of steady-state excretion rates during the continuous-wearing phase for the estradiol transdermal system. The Kruskal-Wallis test was used for determining the effect of treatment order on serum levels of estradiol during the delivery-rate comparison phase.

Results

Pharmacokinetics

Transdermal systems. Mean pretreatment values for serum estradiol and estrone were 7.4 and 32.3 pg/ml, respectively. The three delivery rates for the estradiol transdermal systems (0.025, 0.05, and 0.1 mg/day) raised serum levels of estradiol to 30 to 130 pg/ml within 4 hours of application of the system. After transiently higher levels, serum levels of estradiol were

Table II. Study design and medications

Study week Treatmen:		Duration		
1	No drug (baseline values)	-		
2	Estrace (micronized`17β-estradiol), 2 mg/day orally	3 Days		
3	No drug (baseline values)			
4*	Estraderm, 0.025 mg/day	3 Days) '	
5*	Estraderm, 0.05 mg/day	3 Days	Delivery-rate com-	
6*	Estraderm, 0.1 (2 \times 0.05) mg/day	3 Days	parison phase	
7-9	Estraderm, 0.05 mg/day	Applied twice weekly for 3 weeks	Continuous-wearing	
10	No drug (baseline values)		phase	
11	Premarin (conjugated equine estrogens), 1.25 mg/day orally	3 Days)	
12	No drug	, •		

^{*}Treatment order randomized.

Table III. Mean serum levels and ratios of estradiol and estrone before and during treatment with Estraderm, Estrace, and Premarin

	Before treatment	Estraderm* (mg/day)			Estuace+	Premarin†
		0.025	0.05	0.1	Estrace† (2 mg)	(1.25 mg)
Estradiol (pg/ml)	7.4	23	39	74	66	31
Estrone (pg/ml)	32.3	33	41	59 .	334	152
Estradiol/estrone	0.23	0.70	-0.95	1.25	0.20	0.20

^{*}Mean steady-state values during 72-hour wearing.

maintained at 25, 40, and 75 pg/ml, respectively, during 72-hour wearing. Levels of estrone rose slightly and also remained rather constant, ranging from 30 to 60 pg/ml. Estradiol and estrone both returned to pretreatment levels by 24 hours after removal of the system (Fig. 1, Table III).

Prior to treatment, urinary estradiol and estrone conjugates, expressed in terms of urinary creatinine, measured 0.5 and 2.2 μg/gm, respectively. Values rose through the third day after application of each system, but values for day 3 were not significantly different from those for day 2. Values for estradiol conjugates were in the range of 1.5 to 4.2 μg/gm, and those for estrone conjugates were in the range of 4.6 to 11.3 µg/ gm. By 24 hours after removal of the system, urinary estradiol and estrone conjugates had reached or approached pretreatment levels (Fig. 2).

To determine the relationship between delivery rates and levels of estradiol in serum and urine, we calculated two area under the curve values: (1) for serum estradiol over 72 hours and (2) for urinary estradiol during the interval from application of the system through the end of the third day after removal of the system. The relationship determined by least-squares linear regression analysis of the individual data showed good correlation between delivery rate and area under the curve (Fig. 3).

During continuous wearing of the 0.05 mg estradiol transdermal system, serum levels of estradiol and estrone were similar to those seen in the delivery-rate comparisor, phase of the study (Fig. 4). The mean serum level of estradiol, averaged over the entire 3-week period, was 37.6 pg/ml (± 2.6 SE). Area under the serum estradiol time curve, calculated for one wearing period of each of the 3 weeks, showed no significant differences (p > 0.10). Urinary estradiol conjugates normalized to creatinine were averaged during the third week of system wearing to represent steady state. Values for days 1 to 14 were not significantly different from those for days 15 to 21. As Fig. 5 shows, values were essentially constant during the entire 3 weeks of wearing. Levels approached baseline within 2 days of removal of the last system.

Oral preparations. During oral administration of micronized 17\u00e3-estradiol, mean levels of estradiol in serum were 40 to 60 pg/ml at 24 hours after the third dose, whereas mean levels of estrone were 200 to 340 pg/ml. During the third day of dosing, serum levels of the estrogens were followed more closely. At 8 hours after the oral dose, estradiol and estrone reached à pronounced peak of 133.4 pg/ml (\pm 16.2 SE) and 709.3 pg/ml (±94.5 SE), respectively. Excretion of urinary estradiol conjugates, normalized to creatinine, rose continuously, reaching 55.9 µg/gm (±9.2 SE) after the

[†]Mean values 24 hours after third dose.

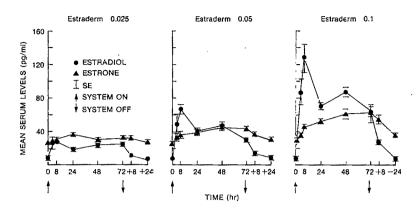


Fig. 1. Mean serum levels of estradio and estrone with use of Estraderm, 0.025, 0.05, and 0.1 mg/day.

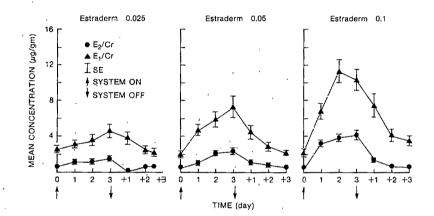


Fig. 2. Mean concentrations of urin 1-1 estradiol and estrone conjugates with use of Estraderm, 0.025, 0.05, and 0.1 mg/day (expressed as μ g/gm of creatinine). E_2 = estradiol; E_1 = estrone; Cr = creatinine.

Table IV. Endogenous estrogen production rates and plasma concentrations of estrogen

		Plasma concentration		
Patient status	Estradiol procestion rate (μg/czy)	Estradiol (pg/ml)	Estrone (pg/ml)	Estradiol/estrone ratio
Premenopausal woman	;			
Early follicular ⁵	65-100	40-60	40-60	0.5-1
Midfollicular ⁶	100-160	60-100		
Late follicular ⁶	320-640	200-400	170-200	1-2
Luteal ⁶	300	190	100-150	1.5
Menopausal woman ^{5, 7, 8}	18	5-20	30-70	0.3-0.8
Man ⁶	30-60	25-45	25-90	0.4-0.6

third day of dosing, and did not return to baselize until 7 days afterward. Urinary estrone conjugates — lowed the same pattern, peaking at 183 µg/gm (±22.3 SE).

Conjugated equine estrogens administered orally produced serum levels of estradiol of about 25 pg/ml at 24 hours after dosing, when estrone was approximately 100 pg/ml. On the third day of dosing peak values of estradiol and estrone at 8 hours were, respectively, 38.9 pg/ml (±4.8 SE) and 196 pg/ml ±21.0

SE). The patterns of urinary estradiol and estrone conjugates normalized to creatinine were similar to the pattern for micronized estradiol, although levels rose more slowly, peaking at 37.9 $\mu g/gm$ (± 10.3 SE) and 91.7 $\mu g/gm$ (± 14.2 SE), respectively. Baseline values were not restored until more than 5 days after the third dose. Figs. 6 and 7 and Table III depict the values attained during oral dosing.

Pharmacodynamics. Serum levels of FSH and LH

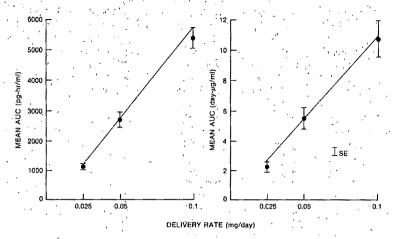


Fig. 3. Mean serum estradiol area under the curve (AUC) (left) and mean urinary estradiol conjugates AUC (right) with Estraderm, 0.025, 0.05, and 0.1 mg/day. ($R^{-2} = -0.97$ and 0.891, respectively.)

(Fig. 8) were lowered by all three forms of estrogen replacement therapy. The estradiol transdermal systems lowered them incrementally in relationship to delivery rate. Compared with pretreatment levels, the reduction was statistically significant (two-tailed, paired t, p < 0.05) in all treatments, except for reduction of LH during continuous wearing of the transdermal system. A comparison of FSH and LH values for each treatment shows that the 2 mg oral dose of estradiol produced an effect equivalent to that of about 0.05 mg/day transdermally.

Serum sex hormone binding globulin was measured at the beginning of the study, just prior to initiation of continuous wearing, and at 24 hours after removal of the last system in the continuous-wearing phase. Mean values increased slightly from a prestudy control value of 1.8 μ g/100 ml (\pm 0.2 SE) to 2.2 μ g/100 ml (\pm 0.1 SE) at 24 hours after removal of the system (two-tailed, paired t, p > 0.1). The posttreatment value was well within the normal range for the laboratory in which the assay was performed. Studies by Geola et al.4 have shown significant elevations in sex hormone binding globulin after 6 weeks of treatment with conjugated equine estrogen at a dose as low as 0.15 mg/day and increasing elevations with higher doses.

Concomitant pharmacologic effects were minimal and essentially the same for each treatment. Such effects included mild tenderness of the breasts and a slight increase in vaginal secretions. The estradiol transdermal systems were well tolerated by the subjects, and the systems adhered well.

Comment

Serum estradiol. Estraderm (estradiol transdermal system) with controlled in vivo delivery rates of 0.025 to 0.1 mg/day provided serum concentrations of estra-

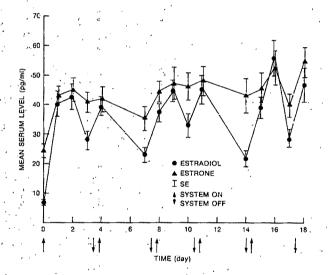


Fig. 4. Mean serum levels of estradiol and estrone during continuous wearing of Estraderm; 0.05 mg/day.

diol typical of the early follicular phase of the premenopausal woman (Table IV) without a substantial increase in serum estrone. The result was a physiologic estradiol/ estrone ratio that approximated 1.

Serum concentrations of estradiol and urinary estradiol conjugates showed dose-proportionality; the three delivery rates augmented the production of endogenous estradiol by factors of two, four, and eight, respectively. Analysis of residual estradiol content of worn systems verified their nominal in vivo delivery rates. For example, the mean difference in residual estradiol content between 247 systems (0.05 mg/day) worn for 72 hours and 245 unused systems was 187 µg (±13.2 SE), which represented in vivo delivery of an average of 62 µg/day, close to the nominal in vivo delivery rate (CIBA-GEIGY, data on file).

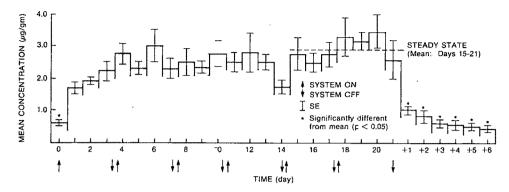


Fig. 5. Mean concentration of urinar estradiol conjugates (expressed as μg/gm of creatinine) during continuous wearing of Estraderm, 0 DE mg/day.

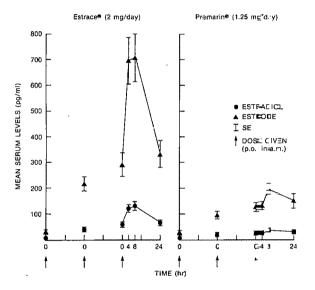


Fig. 6. Mean serum levels of estradiol and estrone vain use of Estrace (2 mg/day) and Premarin (1.25 mg/day).

Continuous wearing of the transdermal estraciol systems over 3 weeks did not result in any accumulation of estradiol. Areas under the curve of serum levels of estradiol were similar in each of the weeks over the continuous-wearing period, and urinary estration conjugates did not increase with time.

Serum levels of estradiol obtained with the transdermal systems were in a range similar to that of those obtained 24 hours after dosing with either cal preparation, although the oral estrogen doses of 1.25 and 2 mg/day substantially exceeded the transdermal input of 0.025 to 0.1 mg/day. Suppression of gonal ctropins by the estradiol transdermal systems was similar to that by the oral preparations; as is well known, however, estrogen replacement therapy does not restor F3H or LH to premenopausal levels.

Serum estrone. Both oral dosage forms mereased serum estrone to levels four to six times the levels of serum estradiol and to seven to 20 times the baseline estrone values. Transdermal input resulted in an ap-

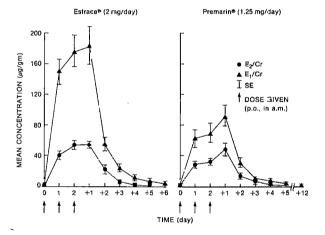


Fig. 7. Mean concentrations of urinary estradiol and estrone conjugates with use of Estrace (2 mg/day) and Premarin (1.25 mg/day) (expressed as μ g/gm of creatinine). E_2 = estradiol; E_1 = estrone; C_1 = creatinine.

proximate doubling of serum estrone and a premenopausal estradiol/estrone ratio.

Urinary conjugates. With oral dosage forms, accumulation of estrone in the body was evident over the 3 days of dosing. This accumulation is more obvious if the kinetics of urinary estradiol and estrone conjugates are taken into consideration. The excretion of urinary estradiol and estrone conjugates reflects the dose relationship of total estrogen administered by oral and transdermal routes. Premarin consists of three major components: estrone sulfate (50% to 60% of the preparation and the only component that contributes to the formation of estradiol) and two equilins (20% to 30% equilin sulfate and 15% dihydroequilin sulfate). Equilins are excreted as conjugates without structural change in the B ring. 9, 10

Estrone sulfate, the major estrogen present after the administration of oral estrogens, has a long biologic half-life because of its enterohepatic recirculation and interconversions with estrone and estradiol.¹¹

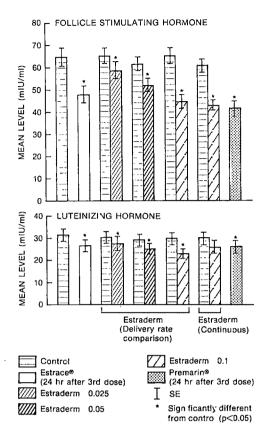


Fig. 8. Comparison of mean serum levels of FSH and LH with use of Estrace, Premarin, and Estraderm.

The plasma kinetics of different conjugates after oral administration of estradiol, compared with those that occur with Premarin, were well analyzed by Schindler et al.,12 who showed that orally administered nonconjugated estradiol and estrone sulfate have a kinetic pattern similar to that shown in this study.

The three transdermal delivery rates produced average levels of urinary estradiol conjugates in the range of 1, 2, and 4 µg/gm of creatinine, respectively. Similar levels of urinary estradiol conjugates (1 to 5 µg/gm) have been measured during the follicular phase in premenopausal women (CIBA-GEIGY, data on file). The oral preparations of 1.25 mg of conjugated equine estrogens and 2 mg of micronized estradiol resulted in levels that peaked at 38 and 55 µg/gm of creatinine, respectively. The relative amounts of urinary estradiol excreted reflect the respective total doses administered (0.025 to 0.1 mg/day transdermally; 1.25 $[\sim 0.7 \text{ mg as}]$ estrone sulfate] to 2 mg/day orally).

Other metabolic effects. With all desage forms, there was no relationship between the decrease in the concentrations of gonadotropins and estrone; in other words, estradiol and not estrone appears to be effective at the pituitary-hypothalamic level. This provides evidence that the high concentrations of estrone and estrone sulfate13, 14 achieved with oral estrogens are not

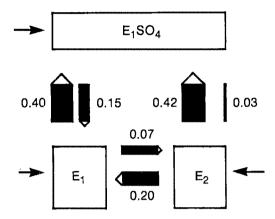


Fig. 9. Interconversions of estrone sulfate (E₁SO₄), estrone (E1), and estradiol (E2). Figures given are [p]38 values, and arrow size is proportional to respective [p] BB values. (Reprinted with permission from Longcope C. The metabolism of estrone sulfate in normal males. J Clin Endocrinol Metab 1972;34:113-

practically effective per se; the estrone and estrone sulfate function as a large precursor pool with slow conversion rate to estradiol¹¹ (Fig. 9).

The oral route of administration for estrogens is, obviously, inefficient and gives rise to a nonphysiologic pattern of metabolites. The high first-pass conversion of estradiol to estrone and estrone sulfate, and the high total amount of estrogens that have to be metabolized by the liver are reflected in nonphysiologic elevation of liver proteins, such as sex hormone binding globulin and other carrier proteins. The most notable of these is renin substrate, a hormone involved in the regulation of blood pressure.4,15 On the other hand, efficacy at the hypothalamic level is probably regulated by estradiol, as evidenced by similar serum levels achieved by the different routes.

The continuous administration of 17β-estradiol afforded by the transdermal formulation was shown previously to control menopausal flushes effectively, as demonstrated objectively by skin thermography. 15 Subcutaneous implants of 17\u03b3-estradiol resulted in similar plasma levels of estradiol and control of hot flushes, which recurred when the levels of estradiol fell below 35 pg/ml.16 The same study also demonstrated that transdermal 17β-estradiol improved vaginal cytologic findings from an atrophic state to a state comparable to that of the follicular phase.

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Failure of Lasix to induce a fetal diuresis

To the Editors

The recent report of Romero et al. (Antenatal diagnosis of renal anomalies with ultrasound. III. Bilateral renal agenesis. Am J Obstet Gynecol 1985; 151;38-43.) included an addendum suggesting that maternal administration of furosemide may not induce fetal diuresis. This addendum contradicted the evidence presented by Wladimiroff indicating a furosemide-induced diuresis based on ultrasound determination of hourly fetal production rates. The fact that Wladimiroff did not use a control population introduces an undefined variability and makes suspect the conclusion. Furthermore, in the study of Kurjak et al. maternal furosemide administration did not result in a significant change in hourly fetal urine production as determined by ultrasound (treated value was 110% ± 24% of controls).

Although the ovine placenta is structurally different from the human placenta, fetal urine production can be monitored directly in the chronically catheterized ovine model. To determine whether furosemide administration to the pregnant ewe affects fetal urine output, two time-dated pregnant ewes of a gestational age of 120 days underwent hysterotomy with insertion of maternal and fetal venous and arterial catheters and fetal bladder catheter (cystotomy). Following 5 days of recovery, fetal urine was drained for 40 minutes to empty the bladder. After a control period of 60 minutes during which fetal urine was collected at 10-minute intervals, furosemide (50 mg) was administered intravenously to the ewe. During the subsequent 60 minutes. we noted no change in fetal urine volume or osmolality. We recognize the preliminary nature of these studies. Nevertheless, in view of the report of Romero et al. we believe that further evaluation of the fetal furosemidediuresis test is necessary before it is accepted as valid.

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Reply

To the Editors:

We would like to thank Drs. Ross, Ervin, and Leake for their interest in our work. Their experimental data confirm the report of Chamberlain et al. indicating the failure of furosemide to augment fetal urinary production when administered to the pregnant ewe. Perhaps this observation can be explained by the poor transplacental crossing of furosemide in the ovine model. Indeed, Chamberlain et al. did not find furosemide in the fetal venous plasma using an assay with a sensitivity of 100 ng/ml. They noted that diuresis could be inconsistently induced even after direct fetal administration of the drug in doses of >3 mg/kg. The value of these interesting observations to human pregnancy remains to be established because there are data to support the view that furosemide does cross the human placenta. Beermann et al.2 have reported cord levels of 330 to 340 µg/ml between 8.5 and 9.5 hours after maternal oral ingestion. However, no drug was detected within the first 4 hours of oral administration.

Our reported experience suggests the potential unreliability of the furosemide test to discriminate between oligohydramnios caused by renal structural abnormalities and that associated with other causes. For these reasons we believe that the test should undergo careful scientific scrutiny in human pregnancy before its continued application in the differential diagnosis of severe oligohydramnios.

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Strict clinicopathologic criteria in the diagnosis of partial hydatidiform mole: A plea renewed

To the Editors:

The article of Drs. Teng and Ballon entitled "Partial hydatidiform mole with diploid karyotype: Report of three cases" (AM J OBSTET GYNECOL 1984;150:961) describes three pregnancies that each manifested a separate fetus with a normal placenta, accompanied by 300 to 400 gm of molar tissue. These conceptuses are not partial moles but are the result of a twin pregnancy in which one of the twins happens to be a complete mole. (The latter seems to be a fair assumption in view of the subsequent clinical history and gross morphology cited

by the authors; unfortunately, histologic appearances are omitted in the paper.) Normal twins with a complete mole are rare, but many examples exist in the literature. An old classical article from 1966 documents 37 cases of single placentas with a normal fetus accompanize by a mole, and a recent, more modern review is pro- ded by Fisher et al.2 These authors also cite a case of their own in which chromosomal analyses proved the totally paternal origin of the complete mole and a normal mother/father origin of the fetus. Nowhere in the literature is the condition described by Teng and Bellon referred to as a partial mole, including the four pazers cited in their article. The partial mole entity was de ned 2 years ago by a World Health Organization scientific group³ as a conceptus with a fetus (alive or dead and a single placenta with focal hydatidiform change cf the villi in the presence of focal (often inconspicuous trophoblastic hyperplasia.

The authors state that "several patients have cereloped malignant sequelae after the diagnosis of incomplete mole" but provide no reference(s) in the literacure to document this statement. While several cases ci residual trophoblastic disease are on record, no unequivocal case of choriocarcinoma has been described to date (Looi and Sivanesaratnam⁵ describe a unique cas≡ in which the patient had a partial mole and was fount to have choriocarcinoma more than 1 year later. **nat makes this case equivocal is that the patient was under no surveillance for some 25 weeks in the interim between the observation of the partial mole and the diagnosis of chorionic cancer). A further statement of the authors that the diagnosis of twin gestation would not alter the clinical management also calls for a comment, because in cases diagnosed late in pregnancy6 a zerefully monitored, short waiting period might possibly allow the normal twin to reach viability.

The purpose of this letter is to plead that both radiologists and obstetricians observe the distinction petween the two molar syndromes and that they take nto account their separate morphology and distinct clinical profiles.7 The occurrence of twinning may obvio_sly add to the usual diagnostic difficulties in molar p-egnancies and calls for special vigilance on the part of sonographer and clinician.

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Reply

To the Editors: .

The entity discussed in our paper is based on the World Health Organization definition of a partial hydatidiform mole, namely, the coexistence of a fetus with a single placenta in which the villi have undergone the changes described. Kajii¹ proposed, based on a study of 1918 induced and spontaneous abortions, that moles be classified into two clinically and histologically distinct categories known as complete moles and partial moles. A partial mole has both hydropic and normal villi and can be associated with an amniotic membrane, umbilical cord, and even a fetus. It has a variety of karyotypes, predominantly triploidy.

There are many references to partial mole requiring chemotherapy after evacuation. (Ref 2, 3, 4) The decision to treat patients with non-metastatic hydatidiform mole is based on a plateau or rise in the serum level of hCG and not on the presence or absence of a coexisting fetus. As we have stated, the possibility of these pregnancies representing twin gestations cannot be excluded; however, the clinician must be aware that mere coexistence of fetus with a molar pregnancy does not obviate the need for diligent monitoring of the serum level of hCG.

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Vulvar effects of the toilet tissue observational routine in the practice of natural family planning

The Creighton University Natural Family Planning Education and Research Center has developed a standardized, objective, technical, case-managed approach to the delivery of natural family planning services.¹ Highly sophisticated, didactic educational processes are used in the training of teachers. Scientific objectivity and reproducibility of results are stressed throughout the I-year course of teacher training. One training tool used to achieve the standardization and reproducibility is the "observational routine" of mucus observation.

This routine of mucus observation consists of having the client wipe the interlabial sulcus of the vulva from the urethra through the perineal body with flat layers of white, unscented toilet tissue to determine the presence or absence of mucus before and after urinating, before and after bowel movements, before going to bed, after bearing down, and before bathing, showering, or swimming. If mucus is present, she is to wipe until the mucus is gone. After wiping, the client observes the toilet tissue. The observation is then noted mentally until the end of the day when the "most fertile sign" of the day is recorded on the chart according to a vaginal discharge recording system. A 100% compliance with this observational routine is considered mandatory. The client following this observational routine of mucus surveillance wipes, in the prescribed manner, an average of 18 times per day.

After I instructed 27 clients who then used the Creighton method, 10 clients (37%) developed either a vulvar irritation or a persistent discharge of mucus or both. Three clients (11.1%) developed only an irritation. Three clients (11.1%) developed only a persistent mucus discharge. Four clients (14.8%) developed both the irritation and the persistent mucus discharge. In every instance the vulvar irritation and/or persistent discharge cleared spontaneously when the client discontinued the tissue-wiping observational routine.

Additionally, in four of the 10 clients exhibiting these problems, who had been wiping correctly for periods of from 3 months to 1 year, a 3 mm punch biopsy was

taken from the medial aspect of the labia minora. These biopsies were examined by a dermatopathologist and revealed histopathologic findings consistent with lichen simplex chronicus.

Toilet tissue is made from processed and chemically treated paper pulp. It is produced by the fining and processing of billions of individual microscopic wood slivers. Dyes are used to obtain color and even to increase its appearance of whiteness. The softness of the tissue is stressed in manufacturer's advertisements for obvious reasons, but because of what it is and how it is made, it is an abrasive.

The purpose of this letter is to alert the profession to a heretofore unreported cause of unexplained vulvar irritation and/or persistent "mucus" discharge that is of vulvar origin, not cervical or vaginal, and strongly appears to be caused by the excessive wiping of the interlabial sulcus of the vulva with an abrasive substance in an observational routine associated with a particular method of natural family planning.

We would be interested in learning whether others have observed similar changes and/or complaints and whether further investigation into this phenomenon is indicated for more precise definition.

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- Postgraduate Course in "The Practice of Colposcopy," October 16-17, 1985, Galvez Hotel, Galveston, Texas. For further information contact: Registration Supervisor, SLACK Inc., 6900 Grove Road, Thorofare, NJ 08086. Tel.: (609) 848-1000.
- Seventeenth Memphis Conference on the Mother, Fetus and Newborn, September 19-20, 1985, The Peabody Hotel, Memphis Tennessee. Sponsored by The University of Tennessee

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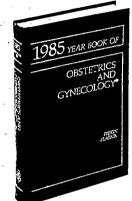
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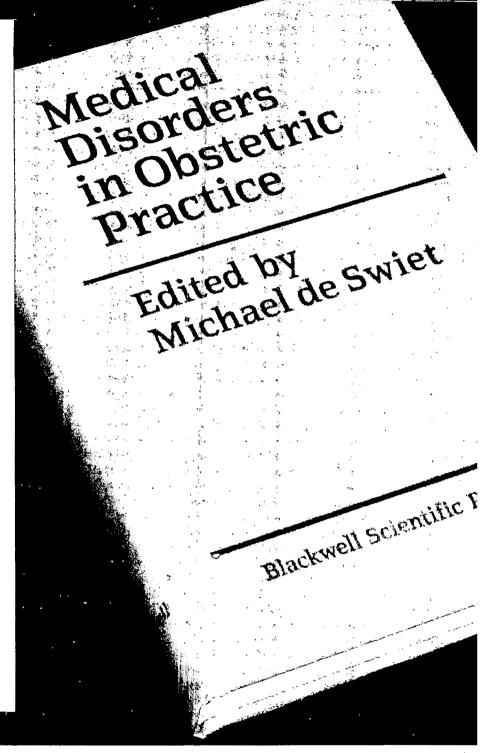
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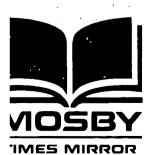
The physiology of the pregnant woman is so altered, and the constraint of the welfare of the fetus is so important, that . . . the two widely different fields of obstetrics and medicine are needed I hope that the obstetrician who may not always have optimal support, will find practical answers to his medical problems here." (Michael de Swiet, from the Preface)

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TRIPHASIL*—6 brown tablets containing 0 050 mg levonorgestrel with 0 030 mg ethinyl estradiol; 5 white tablets containing 0 075 mg levonorgestrel with 0 030 mg ethinyl estradiol; 10 light-yellow tablets containing 0 125 mg levonorgestrel with 0 030 mg ethinyl estradiol; 7 light-green tablets containing inert ingredients are included in the 28-day regimen)—Triphasic regimen indicated for the prevention of pregnancy in women who elect to use oral contraceptives (OC's) as a method of contraception.
Contraindications—OC's should not be used in women with any of the following conditions: 1. Thrombophilebits or thromboembolic disorders. 2. A past history of deep-vein thrombophiebits or thromboembolic disorders. 3. Gerebral-vascular or cororary-artery disease. 4. Known or suspected carcinoma of the breast. 5. Known or suspected estrogen-dependent neoplasia. 6. Undagnosed abnormal genital bleeding 7. Known or suspected estrogen-dependent neoplasia. 6. Undagnosed abnormal genital bleeding 7. Known or suspected estrogen-dependent proplasia. 6. Undagnosed abnormal genital bleeding 7. Known or suspected estrogen-dependent proplasia. 6. Undagnosed abnormal genital bleeding 7. Known or suspected estrogen-dependent proplasia. 6. Undagnosed abnormal genital bleeding 7. Known or suspected estrogen-dependent recognisis. 6. Undagnosed proplasial bleeding 7. Known or suspected estrogen-dependent recognisis. 6. Undagnosed abnormal genital bleeding 7. Known or suspected estrogen-dependent recognisis.

Warnings

Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.

The use of oral contraceptives is associated with increased risk of several serious conditions, including thromboembolism, stroke, myocandal infarction, hepatic adenoma, galibladder disease, hypertension. Practitioners prescribing oral contraceptives should be familiar with the following information relating to

 Thromboembolic Disorders and Other Vascular Problems—An increased risk of thromboembolic and thrombotic disease associated with use of OC's is well established. Three principal studies in Great Britain and 3 in the U.S. have demonstrated increased risk of fatal and nonfatal venous thromboembolism and stroke, both hemorrhagic and thrombotic. These studies estimate that users of OC's are 4 to 11 times more likely than ronusers to develop these es without evident cause

obseases without evident cause.

CEREBROVASCULAR DISORDERS—In a collaborative American study of cerebrovascular disorders in women with and without predisposing causes, it was estimated that the risk of hemorrhagic stroke was 2.0 times greater in users than nonusers and the risk of thrombotic stroke was 4 to 9.5 times greater in users than in nonusers.

than nonusers and the risk of thrombotic stroke was 4 to 9.5 times greater in users than in nonusers MYOCARDIAL INFARCTION (MI)—An increased risk of MI associated with the use of OC's has been reported, confirming a previously suspected association. These studies, conducted in the UK, found, as expected, that the greater the number of underlying risk factors for coronary-artery disease (organete smoking, hypertension, hyper-cholesterolemia, obesty, diabetes, history of pre-ectamptic toxemia) the higher the risk of developing MI, regardless of whether the patient was an OC user or not. OC's, however, were found to be a clear additional risk factor. In terms of relative risk, it has been estimated that OC users who do not smoke (smoking) is considered a meal aso smokers have about a 5-fold increased risk of fatal MI compared to users who do not smoke. OC users who are also smokers have about a 5-fold increased risk of fatal MI compared to users who do not smoke. OC users who are also smokers have about a 5-fold increased risk of fatal MI compared to users who do not smoke. But the standard of the series of the smokers have consideration in mortant factor, in determining importance of these relative risks, however, baseline rates for various age groups must be given serious consideration. Importance of other predisposing conditions mentioned above in determining relatives and absolute risks has not as yet been quantified; quite likely the same synergistic action exists, but perhaps to a lesser extent.

actor. In determining importance of these relative risks, however, baseline rates for various age groups must be given serious consideration. Importance of other predisposing conditions mentioned above in determining relative and absolute risks has not a yet been quantified, quite likely the same synergistic action exists. but perhaps to a lesser extent.

IRSK OF DOSE—In an analysis of data derived from several national adversa-reaction reporting systems. British investigators concluded that risk of thromboembolism, including corrovary thrombosis, is directly related to dose of estrogen in 0°Cs. Preparations containing 10°D mongor more of estrogen were associated with higher risk of informment of the properties of the properties of the properties of the properties of the service of the strong of the properties of the properties

- with breast nodules, fibrocystic disease, or abnormal mammograms should be monitored with particular care if they elect to use OC's

 4. Hepatic Tumors—Benigh hepatic adenomas have been found to be associated with use of OC's. One study showed that OC's with high hornoral potency were associated with higher risk than lower potency OC's. Although benigh, hepatic adenomas may ruptize and may cause death through intra-abdominal hemorrhage. This has been reported in short-term as well as long-term users. Two studies relate risk with duration of use of OC's, the risk being much greater after 4 or more years use. While hepatic adenoma is rate, it should be considered in women presenting abdominal pain and tenderness, abdominal mass or shock. A few cases of hepatocellular carcinoma have been reported in women on OC's. Relationship of these drugs to this type of malignancy is not known.

 5. Use in or immediately Preceding Pregnancy Birth Defects in Offspring, and Malignancy in Female Offspring—Use of temale sex hormones—both estrogenic and progestational agents—curing early pregnancy may seriously damage the offspring. It has been shown that females exposed in utero to disthylstibestrol, a nonsteroidal estrogen, have increased risk of developing in later life a form of vaginal or cervical cancer ordinarily extremely rare. This risk has been estimated to be of the order of 1 in 1.000 exposures or less. Although there is no evidence on what OC's further enhance risk of developing this type of malignancy, such patients should be monitored with particular care if they elect to use OC's Furthermore, 30 to 90% of such exposed women have been found to have epithelial changes of the vagina and cervix. Although these changes are histologically benigh, it is not known whether this condition is a precursor of vaginal malignancy. Male children so exposed my develop abnormalities of the urgenital tract. Although similar data are not available with use of other estrogens, it cannot be presumed they would not induce similar changes. A

tests for pregnancy, or attempted treatment for threatened abortion). Some exposures involved only a few days. Data suggest that risk of himb-reduction defects in exposed fetuses is somewhat less than 1 in 1 000 live births. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well-controlled studies that progestogens are effective. There is some evidence that triplicidy and possibly other types of polyploidy are increased among abortuses from women who become pregnant stoon after casing OC's. Embryos with these anomalies are virtually always aborted spontaneously. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping OC's is unchown. Its recommended that for any patient who has missed 2 consecutive periods, pregnancy should be ruled out a before continuing OC's. It they altered should be prospected to the prescribed schedule, the possibility of pregnancy should be considered at time of first missed period, and further use of OC's should be withheld until pregnancy has been ruled out. If pregnancy is confirmed, the patient should be apprised of the potential risks to the lettus, and advisability of continuation of the pregnancy should be critically an exposition of the pregnancy should be critically an exposition of the pregnancy should be discussed it is also recommended that women who discontinue OC's with intent of becoming pregnant use an attential form of contraception of a period of time before attempting to conceive Many clinicals recommended biseases in uses of ocitical singular pregnancy should be discussed in a state of the patients of

mothers on OC's; effects, if any, on the breast-fed child have not been determined. If feasible, deter OC's until infant has been weaned

Precarutions—GENERAL—1. A complete medical and family history should be taken prior to initiation of OC's.

Pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, subdomen and pelvic organs, including Pap smear and relevant laboratory takes. As a general rule OC's should not be prescribed for longer than 1 year without another physical examination and Pap smear.

2. Under influence of estrogen-progestogen preparations, preexisting uterine leiomyomata may increase in size.

3. Patients with history of psychic depression should be carefully observed and the fung discontinued if depression recurs to a serious degree. Patients becoming significantly depressed while on OC's should stop OC's and use an attendate method to try to determine whether the symptom is drug related.

4. OC's may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggregated by fluid retention, such as convulsive disorders, migraine syndrome; astimate, or cardiace for renal insufficiency.

5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence while on OC's. If jaundice develops, OC's should be discontinued.

6. Staroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution.

7. OC users may have disturbances in normal tryptophan metabolism which may result in a relative pyridoxine.

with carlion.

7. GC users may have disturbances in normal tryptophan metabolism which may result in a relative pyridoxine distinction. Clinical significance is undetermined.

8. Serum foliate levels may be depressed by QC's. Since the pregnant woman is predisposed to development of totale deficiency and incidence of foliate deficiency increases with increasing gestation, it is possible that if a woman becomes pregnant shortly after stopping QC's, she may have a greater chance of developing foliate deficiency and complications attributed to this deficiency.

9. The pathologist should be advised of QC therapy when relevant specimens are submitted.

10. Certain enddering and liver-function tests and blood components may be affected by estrogen-containing QC's.

at horeased sulforcerophihalein retention.

Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3: increased norepinephrine-induced platelet aggregability.

Increased tyroid-binding globulin (TBG) leading to increased circulating total-thyroid hormone, as measured by protein-bound iodine (PBI), 14 by column, or 14 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG free 14 concentration is unaltered.

d. Decreased pregnanediol excretion

e. Reduced response to metyrapone test

Reduced response to metyrapone test Information for the Patient—See Patient Package Labeling.

 Drug Interactions—Reduced efficacy and increased incidence of breakthrough bleeding have been associated with concomitant use of trifampin. A similar association has been suggested with barbiturates, phenylbutazone, phenyton sodium ampiculin and tetracycline.

 Carcinogenesis—See Warnings section for information on carcinogenesis.

 Pregnacy—Category X. See Contraindications, Warnings.

 Nursing Mothers—See Warnings

 Adverse Reactions—An increased risk of these serious adverse reactions has been associated with use of OC's (see Warnings). Intrombophishis, pulmonary embolism, coronary thrombosis, cerebral thrombosis, cerebral hemorphage, hypertension, galibladder disease, benigh hepatomas, congenital anomalies. There is evidence of an association between the following conditions and use of OC's although additional confirmatory studies are needed mesenteric thrombosis, neuro-ocular lesions, e.g., refinal thrombosis and optic neuritis.

The following adverse-reactions have been reported in patients on OC's and are believed to be drug-related. Nausea and/or vomiting, usually the most common adverse reactions, occur in approximately 10 percent or less of patients during the first cycle. Other reactions, as a general rule, are seen much less frequently or only occasionally castionality in the first cycle. Other reactions, as a general rule, are seen much less frequently or only occasionally membrane of terament, elements of the dema; chlosama or melasma which may persist, breast changes tendemess, enlargement, and secretion, change in weight (increase or decrease), change in cervical erosion and cervical secretion; possible diminution in lactation when given immediately postpartum; cholestatic jaundice, imgraine increase in size of uterine leiomy-omata, rash (allerge); mental depression; reduced tolerance to carbohydrates; vaginal candidiasis; change in users of OC's and the association has been neithe

Acute Overdose—Serious ill effects have not been reported following acute ingestion of large doses of OC's by young children. Overdosage may cause nausea, and withdrawal bleeding may occur in females. Ossage and Administration—For maximum contraceptive effectiveness, Triphasil must be taken exactly as directed and at intervats not over 24 hours. (If Triphasil is first taken later than first day of first menstrual cycle of medication or postpartum, contraceptive reliance should not be placed on it until after the first 7 consecutive days of use. Possibility of ovulation and conception prior to initiation of medication should be considered.)

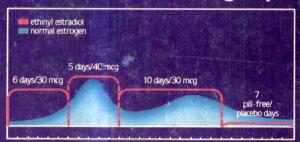
Anytime patient misses 1 or 2 brown, while or light-yellow tablets, she should also use another contraceptive method until she has taken a tablet daily for 7 consecutive days. For full details on dosage and administration see prescribing information in package insert.



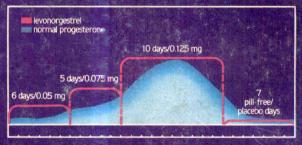
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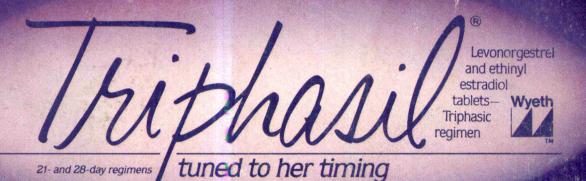


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rious as well as minor adverse reactions have en reported following the use of all oral contraceptives.

See important information on adjacent page.

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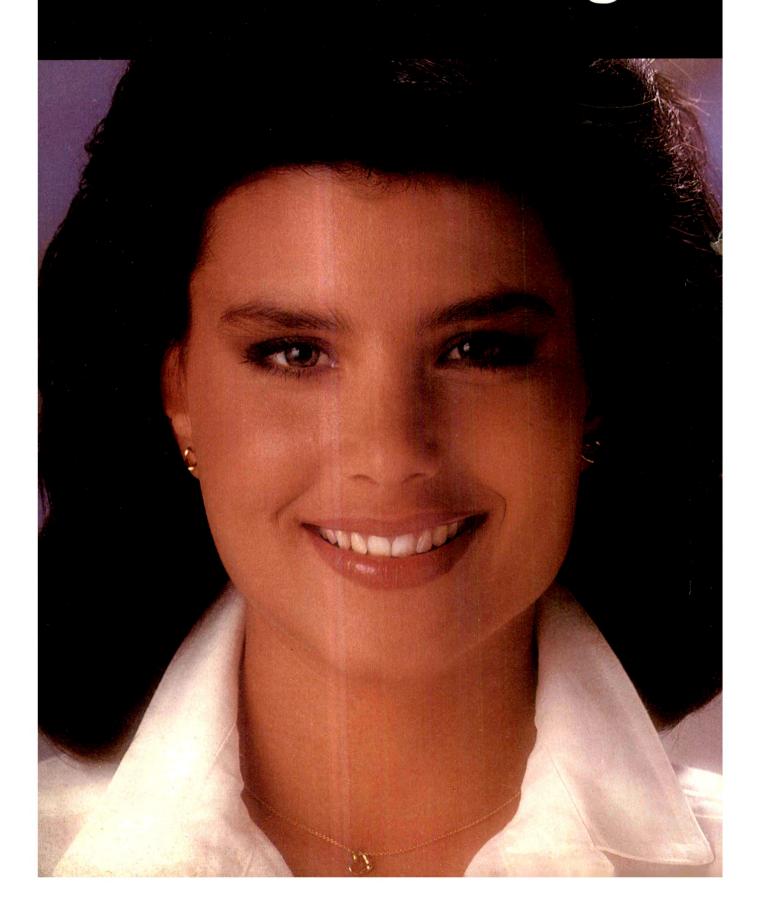
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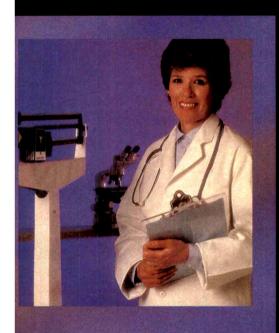
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- 1. 1/35 formulations contain 1.0 mg norethindrone with 0.035 mg ethinyl estradiol. BTB comparisons are based on the first three cycles of use. Data available from Syntex Laboratories, Inc.
- Wynn V, Niththyananthan R: The effect of progestins in combined oral contraceptives on serum lipids with special reference to high-density lipoproteins. Am J Obstet Gynecol 142:766-772, 1982.
- Wynn V: Effect of duration of low-dose oral contraceptive administration on carbohydrate metabolism. *Am J Obstet Gynecol* 142:739-746, 1982.
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green tablet contains norethindrone 10 mg with ethinyl estradiol 0.035 mg.)

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BREVICON* 28-Day Tablets (21 norethindrone 0.5 mg with ethinyl estradiol 0.035 mg.)

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NORINYL* 1 + 35 2.08 Tablets (21 norethindrone 1 mg. with ethinyl estradiol 0.035 mg.)

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writin mestranol Q.D.5 mg, tablets followed by 7 inert tablets)

NORINYL* 1 + 80 21-Day Tablets (norethindrone 1 mg, with
mestranol 0.08 mg),
NORINYL* 1 + 80 28-Day Tablets (21 norethindrone 1 mg,
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mestranol 0.1 mg

WARNINGS: Cigarette smoting increases the risk of serious cardiovascular side affects from OC use. This risk increases with age and with heavy smotling (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use OCs should be strongly advised not to smoke.

The use of OCs is associated with increased risk of several serious con-ditions including thromboembolism, stroke, myocardial infarction liver tumor, gall bladder disease, visual disturbances, letal abnormalities, and hypertension. Practitioners prescribing OCs should be familiar with the following information relating to these risks.

The use of Ocs is associated with increased risk of several serious conditions including thromboembolism. Strike, myocardial infarction liver tumor, gall bladder disease, visual disturbances, fetal abnormalities, and hypertension. Practitioners prescribing OCs should be familiar with the following information relating to these risks.

1. Thromboembolic Disorders and Other Vascular Problems: An increased risk of thromboembolic and thrombolic disease associated with OC use is established. One study demonstrated an increased relative risk for fatal venous thromboembolism and several studies demonstrated for non-fatal venous thromboembolism. They estimate that OC users are 4-11 times more likely than nonusers to develop these diseases without evident cause. One British study reported an excess Gath rate of 40% in OC users, most of which resulted from controls an accrease Gath rate of 40% in OC users, most of which resulted from controls an accrease Gath rate of 40% in OC users, most of which resulted from controls and controls, an accreased relative risk for strike and section was additionally significant. A U.S. prospective study falled was sectionally attentions of the strike of the strike

mortality is with the condom or diaphragm backed up by early abortion. The study also concluded that OC users who smoke, especially over 30, have greater mortality risk than OC users who do not smoke.

Table 1. Risk of thromboembolic and thrombolic disease associated with OCs increases with age after 30 and, for MI, is further inweased by hypertension, hyperhipdemias, owenty, diabetes, or history of precdamplic toxemia, and especially by smoking. The following chart gives a gross estimate of risk of death from circulatary disorders associated with OC use.

SMHCKING HABITS AND OTHER PREDIBIPOSING CONDITIONS—RISK ASSOCIATED WITH USE OF OCS.

300 01 703			
Ag∈	Below 30	30-39	40+
Heavy smokers	С	В	Α
Light smokers	D	Č	P
Nonsmokers	-	-	-
(no predisposing conditions)	n	0.0	r
Nonsmokers	•	0,0	
(other predisposing conditions)	С	C,8	B.A

Use associated with very high risk.
 Use associated with high risk.
 Use associated with moderate risk.
 Use associated with low risk.

Deputs and optient should be alert to earliest manifestations of thromboempolice and thromboem clisorders (e.g., thrombophiebles, pulmonary embosion,
cerebrovascular insufficiency, accornary occlusor, ertanal thromboems and
mesenteric thromboss). Should any of these occur or be suspected, discontionation of the control of the cont

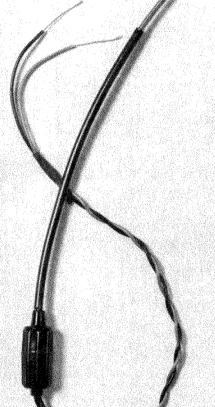
out pregnancy before continuing the OC. If pregnancy is continued, tell the patient about potential risks to the fetus and discuss advisability of continuing the pregnancy Women and discussional COs to become pregnant should use an experiment of the pregnancy Women and the continued COs to become pregnant should use an experiment of the continued of the continu

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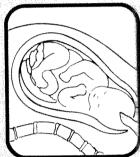


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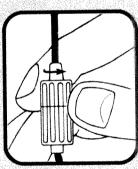
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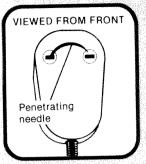




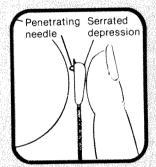
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3. Press the electrode needle firmly against



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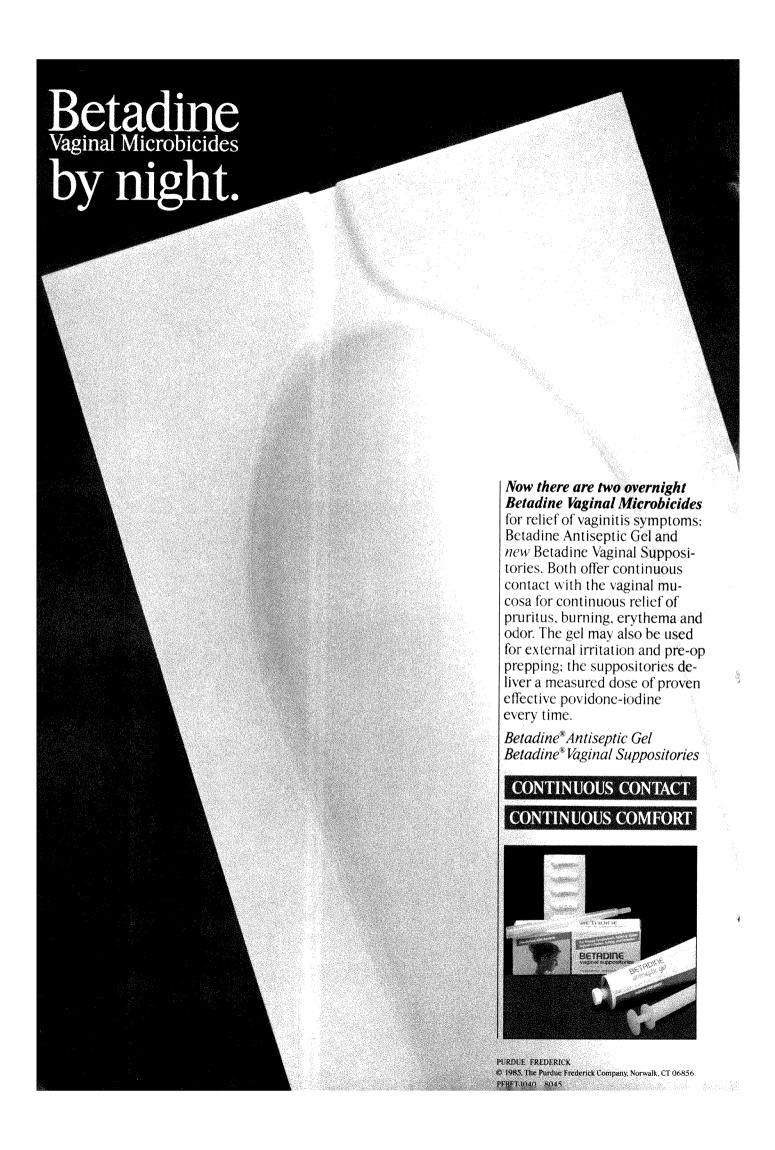
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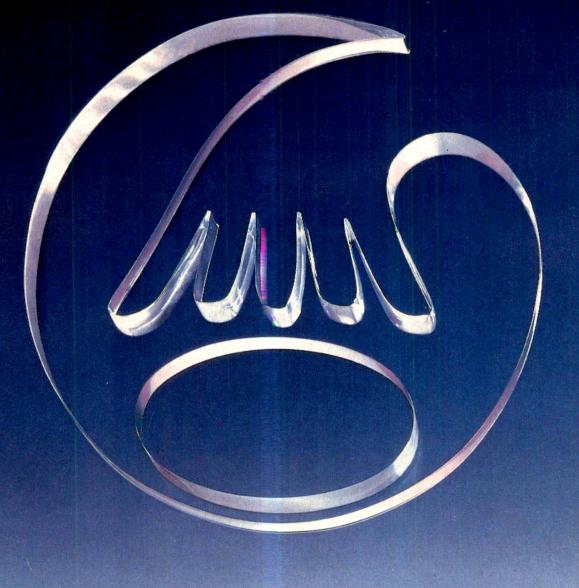
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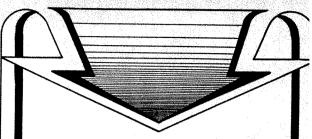
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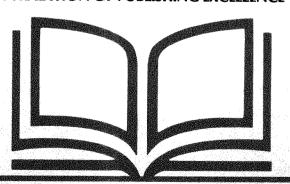
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Editorial policies

The requirements for manuscripts submitted to the American Journal of Obstetrics and Gynecology conform to the "Uniform Requirements for Manuscripts Submitted to Biomedical Journals" established by the International Committee of Medical Journal Editors and published in Annals of Internal Medicine 1982;96(6):766-71. Certain requirements unique to our JOURNAL are provided in Information for Authors, published in each issue of the JOURNAL, and in more detail in the Guide to Writing for the American Journal of Obstetrics and Gynecology. The latter may be obtained from The C. V. Mosby Company or the Editors on request.

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It is assumed by the Editors that articles emanating from a particular institution are submitted with the approval of the requisite authority. Articles dealing with human experimentation that require local institutional approval must have this approval prior to submission of the article for publication and it should be so indicated in the Methods section. Reports of experiments on animals must indicate which guidelines for the care and use of the animals were followed.

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The original and two good-quality copies of the manuscript and three glossy prints of illustrations are required.

E. J. Quilligan, Editor

Department of Obstetrics and Gynecology, University of California. Davis, School of Medicine, Professional Bldg., 4301 X St., Sacramento, CA 95817

Albert B. Gerbie, Associate Editor
710 North Fairbanks Court, Chicago, IL 60611

Manuscripts must be typed double spaced on one side only on 22 by 28 cm (8 1/2 by 11 inch) white bond paper with 1 inch margins at top, bottom, and sides. Number pages consecutively in the upper right-hand corner in the following order: title page, abstract, body of text, references, legends, and tables.

Title page. The title page should contain in sequence the title (concise and suitable for indexing purposes); author line with first name, middle initial, and last name of all authors and their highest academic degree (both M.D. and Ph.D. are acceptable); city(ies), state(s) in which the study was conducted; departmental, divisional, and institutional affiliations at the time the study was performed; acknowledgement of source(s) of support; presented line if applicable; disclaimers, if any; name and address of author to whom requests for reprints should be addressed (if reprints will not be available, it should be so stated); and name and address of author responsible for correspondence concerning the manuscript if different from author to whom reprint requests are addressed. At the bottom of the title page supply a short title for the running head not exceeding 52 characters (including word spaces).

Abstract page and key words/phrases. On manuscript page two is the abstract, also typed double spaced with the required margins and headed by the title of the article and author(s) name. Abstracts for regular articles, Current Investigation, Clinical Opinion, and Current Development may not exceed 150 words. Abstracts for case reports and brief communications may not exceed 50 words. Below the abstract list 3 to 5 key words or short phrases for indexing purposes.

Text. Do not hesitate to write your manuscript in the firstperson, active voice if it is more appropriate to the information you wish to convey. The passive voice is generally more effective for describing techniques or observations since the emphasis is on the "action" rather than the person performing the action.

Only standard abbreviations are to be used. Consult the Council of Biology Editors Style Manual or the AMA's Manual for Authors and Editors. Abbreviations in the title are not acceptable. They should be avoided, if possible, in the abstract. In the text they should be kept to a practical minimum. The full term for which an abbreviation stands should precede its first use in the text unless it is a standard unit of measurement.

Either the generic, chemical, or proprietary names of drugs may be used. If the generic or chemical name is used, authors may, if they desire, insert the proprietary name in parentheses after the first mention in the text, with the name of the manufacturer and city and state.

Regular articles are customarily organized into the following sections: an introduction and headings that identify Material and Methods, Results, and Comment. Authors may wish to summarize their findings in a short paragraph at the end of the Comment section. This format may not be appropriate for some types of articles.

In the introduction, state concisely the purpose and rationale for the study and cite only the most pertinent references as background.

In the Material and Methods section describe briefly (but in sufficient detail to permit other workers to evaluate and reproduce the results) the plan, patients and/or experimental animals and controls, methods and procedures utilized, and statistical method(s) employed.

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Acknowledgements. Acknowledge only persons who have made substantive contributions to the study.

References. Limit of 16 references, except for case reports and brief communications, limited to 2, and Current Development, for which there is no limit. Number references consecutively in the order in which they are mentioned in the text. Use the Drmat of the U.S. National Library of Medicine in Cumulated Index Medicus. Journal titles should also conform to abbreviations used in Cumulated Index Medicus.

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Requirements for special sections

Case reports and brief clinical and basic science communications. Limit of 700 words, 2 references. Include abstract of 50 words maximum, 3 to 5 key words/phrases for indexing purposes, and short title. If tables and/or figures or used, an equivalent number of words must be deducted from the total (see "Estimating Length of Manuscript").

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of \$45.00 U.S. and the fee must accompany the request to publish. Information will be limited to title of meeting, date, place, and an address to obtain further information. Send announcements and payment, payable to this JOURNAL, to The C. V. Mosby Company, 11830 Westline Industrial Drive, St. Louis, Missouri 63146.

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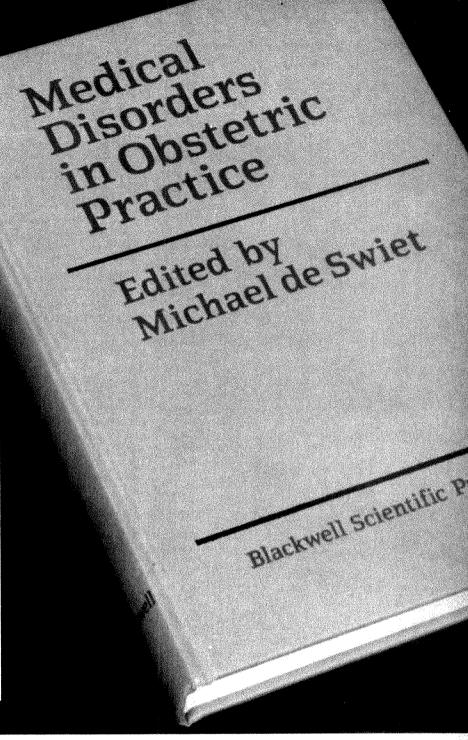
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1. Rössner S et al: Acta Obstet Gynecol Scand 59:255, 1980.
2. Nash AL et al: Med J Aust 2:277, 1979. 3. Larsson-Cohn U et al: Fertil Steril 35:172, 1981. 4. Briggs MH: J Reprod Med 28:92, 1983. 5. Åhrén T et al: Contraception 24:451, 1981.
6. Briggs MH, Briggs M: Acta Obstet Gynecol Scand Suppl 105:25, 1982.

Serious as well as minor adverse reactions have been reported following the use of all oral contraceptives

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Clearette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use that contraceptives should be strongly advised not to smoke. The use of oral contraceptives is associated with increased risk of several serious conditions, including thromboembolism, stroke, myocardial infarction, hepatic adenoma, galibladder disease, hypertension. Practitioners prescriping oral contraceptives should be familiar with the following information relating to these risks

Thromboembolic Disorders and Other Vascular Problems—Ad Intrompoembour Ussorders and Other Vascular Problems—An increased risk of thromboembolic and thrombotic disease associated with use of OC's is well established. Three principal studies in Great Britain and 3 in the U.S. have demonstrated increased risk of fatal and nonfatal venous thromboembolism and stroke, both hemorrhagic and thrombotic. These studies estimate that users of OC's are 4 to 11 times more likely.

These studies estimate that users of OC's are 4 to 11 times more likely. These studies estimate that users of OC's are 4 to 11 times more likely than nonusers to develop these diseases without evident cause CEREBROVASCULAR DISORDERS—In a collaborative American study of cerebrovascular disorders in women with and without predisposing causes, it was estimated that the risk of hemorrhagic stroke was 2.0 times greater in users than nonusers and the risk of thrombotic stroke was 4 to 9.5 times greater in users than in nonusers.

MYOCARDIAL INFARCTION (MI)—An increased risk of MI associated with the use of OC's has been reported, confirming a previously suspected association. These studies, conducted in the UK, tound, as expected, that the greater the number of underlying risk factors for conarry-artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of pre-eclamptic toxemia) the higher the risk of developing MI, regardless of whether the patient was an OC user or not. OC's, however, were found to be a clear additional risk factor. In terms of relative risk, it has been estimated that OC users who do not smoke (smoking is considered a major predisposing condition to MI) are about twice as likely to have a fatal MI as nonusers who do not smoke (Cusers who da real sos mokers have about a 5-fold increased risk of fatal MI compared to users who do not smoke, but about a 10- to 12-fold OU users who are also smokers have about a 5-fold increased risk of fatal MI compared to users who do not smoke, but about a 10- to 12-fold increased risk compared to nonusers who do not smoke. Furthermore, amount of smoking is also an important factor. In determining importance of these relative risks, however, baseline rates for various age groups must be given serious consideration. Importance of other predisposing conditions mentioned above in determining relative and absolute risks has not as yet been quantified; quite likely the same synergistic action exists, but perhaps to a lesser extent. RISK OF DOSE—In an analysis of data derived from several national adverse association and the property of the pro

HISK OF DUSE—In an analysis of data derived from several national adverse-reaction reporting systems. British investigators concluded that risk of thromboembolism, including coronary thrombosis, is directly related to dose of estrogen in OC's. Preparations containing 100 mcg or more of estrogen were associated with higher risk of thromboembolism than those containing 50-80 mcg. Their analysis did suggest, however, that quantity of estrogen may not be the sole factor involved. This finding has been confirmed in the U.S.

ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASES—A large prospective study carried out in the ILK sectionated the procedure.

ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASES—Large prospective study carried out in the UK estimated the mortatility rate per 100.000 women per year from diseases of the circulatory system for users and nonusers of OC's according to age, smoking habits, and duration of use. Overall excess death rate annually from circulatory diseases for OC users was estimated to be 20 per 100.000 (ages 15-34—5/100,000; ages 35-44—33/100,000; ages 45-49—140/100,000), risk being concentrated in older women, in those with long duration of use, and in cigarette smokers. It was not possible, however, to examine interrelationships of age, smokens, and direction of use in compared. 5/100,000: ages 35-44—33:100,000: ages 45-49—140:100,000), risk being concentrated in older women, in those with long duration of use, and in cigarette smokers. It was not possible, however, to examine interrelationships of age, smoking, and duration of use, nor to compare effects of continuous use intermittent use. Although the study showed a 10-fold increase in death due to circulatory diseases in users for 5 or more years all these deaths occurred in women 35 or older. Until larger numbers of women under 35 with continuous use for 5 or more years are available, it is not possible to assess magnitude of relative risk for this younger group. Available data from a variety of sources have been analyzed to estimate risk of death associated with various methods of contraceptive method (e.g., thromboembolic and thrombotic disease in the case of OC's) plus risk attributable to pregnancy or abortion in event of method failure. This latter risk varies with effectiveness of method. The study concluded that mortality associated with all methods of birth control is low and below that associated with childbirth, with the exception of OC's in women over 40 who smoke. Lowest mortality is associated with condom or diaphragm backed up by early abortion. Risk of thromboembolic and thrombotic disease associated with OC's increases with age after about 30 and, for MI, is further increased by hypertension, hyper-cholesterolemia, obesity, diabetes, or history of pre-celamptic toxemia, and especially cigarette smoking. Physician and patient should be alter to earliest manifestations of thromboembolic and thrombotic disorders. If feasible, OC's should be discontinued in mediately. A 4 to 6-fold increased risk of postsurgery thromboembolic complications has been reported in OC users. If feasible, OC's should be discontinued at least 4 weeks before surgery of a type associated with increased risk of thromboembolism or proforinged immobilization.

PERSISTENCE OF RISK OF VASCULAR DISORDERS—Findings from one study in Britani involving ce

3. Carcinoma-Long-term continuous administration of either natural

of synthetic estronen in certain animal species increases frequency of cardinoma of the creast, cervix, vagina, and liver. Certain synthetic progestogens, note currently contained in OC's, have been noted to increase incidence of mammary nodules, beingh and matignant, in dogs progestogens, nore currently contained in UC.s. have been noted to increase incidence of mammary indules, beingn and malignant in dogs. In humans, 3 case-control studies have reported an increased risk of endometrial carcinoma associated with prolonged use of exogenous estrogen in postmencausal women. One publication reported on the first 21 cases submitted by physicians to a registry of cases of adenocationary of the endometrium in women under 40 on OC's. Of cases found in women withourspresisposing risk factors (e.g., irregular bleeding at the time-OC's were first given, polycystic ovaries), nearly all occurred in women withourspresisposing risk factors (e.g., irregular bleeding at the time-OC's were first given, polycystic ovaries), nearly all occurred in women who had used a sequential OC. These are no longer marketed. No evidence has been reported suggesting increased risk of endometrial cancer in users of conventional combination or progestogen-only OC's. Several studies have round no increase in breast cancer in women taking. OC's or estrogene. One study, however, white also noting no overall increased risk of real-t cancer in women on OC's, found an excess risk in subgroups of GD users with documented beingo breast disease. Reduced occurrence of beingo breast timors in users of OC's has been well documented in summary, there is at present no confirmed evidence from human studies of increased risk of cancer associated with OC's. Close clinical surveillance of all women on OC's is, nevertheless, essential. In all cases of unbagnosed persistent or recurrent abnormal vaginal bleeding, appropriate lagnostic measures should be taken to rule out malignancy. Women with a strong family history of breast cancer or with hiseast nodules, firancostatic disease, or abnormal mammograms should be monitored with partic lar care if they elect to use OC's.

Hepatic fumor:—Beingin hepatic adenomas have been reported in short-term as well as long-t-trin users. Two studies relate risk with duration of use of OC's, the risk b

cases of hepatocerular carcinoma have been reported in women of Relationship of these drugs to this type of malignancy is not known.

senting addomina pain and tenderness, abdominal mass or shock. A few cases of hepatocerular carcinoma have been reported in women on OC's Relationship of these drugs to this type of malignancy is not known.

5. Use in or Immediately Preceding Pregnancy, Birth Defects in Offspring, and Malignancy is Fernale Offspring—Use of female sex hormons—both estrogenic and progestational agents—during early pregnancy may seriously damage the offspring. It has been shown that females exposed in utero to diethy stibisshol, a nonsteroidal estrogen, have increased risk of developing in liber afe a form of vaginal or cervical cancer ordinarily extremely rare. This insk has been estimated to be of the order of 1 in 1,000 exposures or less. Although there is no evidence now that OC's further enhance in the Celevioping this type of malignancy, such patients should be monitored with particular care if they elect to use OC's. Furthermore. 30 to 59% of such exposed women have been found to have epithelial changes. In the vagina and cervix. Although these changes are histologically bensin. It is not known whether this condition is a precursor of vaginal malignancy. Male children so exposed may develop abnormalities of the uncigenital hact. Although similar data are not available with use of other estrogenic in the children so exposed may develop abnormalities of the uncigenital hact. Although similar data are not available with use of other estrogenic in the defects, has been reported with use of sex hormones, including OC's, in pregnancy. One case-control study estimated a 4.7-fold increase as risk of limb-reduction defects in infants exposed in utero to sex hormone. (CC's, hormonal withdrawal tests for pregnancy or attempted treatment for threatmend abortion). Some exposures involved only a few days. Data suggest that risk of limb-reduction defects in exposed fetuses is onewhat less than 1 in 1,000 live births. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatment of the treatment for the OC's. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at time of first missed period, and further use of:DC's should be withheld until pregnancy has been ruled out. If pregnancy is confirmed, the patient should be apprised of the potential risks to the fetus, and advisability of continuation of the pregnancy should be esscussed. It is also recommended that women who discontinue OC's with intent of becoming pregnant use an alternate form of contraception for a period of time before attempting to conceive. Many clinicians recommend 3 months, although no precise information is available on which to base this. The administration of progestogen-estroger complianting to include which result is entired. combinations to issues withdrawal bleeding should not be used as a

Combinations of pregnancy of pregnancy 5. Studies report increased risk of surgically confirmed gallbladdemissrase in users of OC's and estrogens. In one study, increased risk appeared after 2 years use and doubled after 4 or 5 years use. In one of the other studies, increased risk was apparent between 6

use. In one of the other studies, increased risk was apparent between 6 and 12 months use. 7 Carbohydrate and Loid Metabolic Effects—Decrease in glucose tolerance has been observed in a significant percentage of patients on OC's. For this reason, prediabetic and diabetic patients should be carefully observed while on OC's. Increase in triglycerides and total phospholipids has been observed in autients on OC's clinical significance of this finding remains to be defined. 8 Elevated Blood Pressure—Increase in blood pressure has been reported in patients on OC's in some women, hypertension may occur

8 Elevated Blood Pressure—Increase in blood pressure has been reported in patients on OC's. In some women, hypertension may occur within a few montes or beginning OC's. In the 1st year of use, prevalence of women with hypertinision is low in users and may be no higher than that of a compariar is group of nonusers. Prevalence in users increases, however, with longer explosure, and in the 5th year of use is 2½ to 3 times the reporteciprevalence in the 1st year. Age is also strongly correlated with development of hypertension in OC users. Women who previously have had hyperthisoin during pregnancy may be more likely to develop elevation at bibod pressure on OC's. Hypertension that develops as a result of taking OC's usually returns to normal after discontinuing the drug.

as a result of taking 0° s usually returns to normal after discontinuing the drug.

9. Headache—Onset er exacerbation of migraine or development of headache of a new pathern which is recurrent, persistent, or severe, requires discontinuation of °°° C's and evaluation of the cause.

10. Bleeding Irreginariuse—Breakthrough bleeding, spotting, and amenorrhea are frequent reasons for patients discontinuing 0°C's. In breakthrough bleeding, as in all cases of irregular vaginal bleeding, normunctional causes shound the borne in mind. In undiagnosed persistent or recurrent abnormal blieding from the vagina, adequate diagnostic measures are indicates to sile out pregnancy or malignancy (f pathology has been excluded, time or change to another 0°C may solve the problem Changing to an 0°C wim a higher estrogen content, while potentially useful

in minimizing menstrual irregularity, should be done only if necessary, since this may increase risk of thromboembolic disease. Women with past history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may have a tendency to remain anovulatory or to become amenorrheic after discontinuing OC's. Women with these preexisting problems should be advised of this possibility and encouraged to use other methods. Post-use anovulation, possibly prolonged, may also occur in women without previous irregularities.

11. Ectopic Pregnancy—Ectopic as well as intrauterine pregnancy may occur in contraceptive failures.

12. Breast-feeding—OC's given in the postpartum period may interfere with lactation and decrease quantity and quality of breast milk. Furthermore, a small fraction of the hormones in OC's has been identified in the milk of mothers on OC's, effects, if any, on the breast-fed child have not been determined. If feasible, defer OC's until infant has been weened. Precautions—GENERAL—1. A complete medical and family history should be taken pror to intation of OC's. Pretreatment and periodic physical examinations should include special reference to blood pressure. breasts, abdomen and pelvic organs, including Pap smear and relevant laboratory tests. As a general rule OC's should not be prescribed for longer than 1 year without another physical examinations.

2. Under influence of estrogen-progestogen preparations, preexisting uterine leionyomata may increase in size.
3. Patients with history of psychic depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. Patients becoming significantly depressed while on OC's should stop CC's and use an alternate method to try to determine whether the symptom is drug-related.
4. OC's may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, asthma, or cardiac or renal insufficiency. insufficiency.

Patients with a past history of jaundice during pregnancy have an increased risk of recurrence while on OC's. If jaundice develops, OC's

indicased risk of recurrence while on OC s. If jaundice develops, OC's should be discontinued.

6. Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution.

7. OC users may have disturbances in normal tryptophan metabolism.

which may result in a relative pyridoxine deficiency. Clinical significance is undetermined

undetermined

8. Serum folate levels may be depressed by OC's. Since the pregnant woman is predisposed to development of folate deficiency and incidence of folate deficiency increases with increasing gestation, it is possible that if a woman becomes pregnant shortly after stopping OC's, she may have a greater chance of developing folate deficiency and complications attributed to this deficiency.

Internation for the Patient—See Patient Package Labeling.

Laberatory Tests—1. The pathologist should be advised of OC therapy when relevant specimens are submitted.

Certain endocrops and their functions.

Laboratory Tests—1. The pathologist should be advised of UL merapy when relevant specimens are submitted.

2. Derfain endocrine—and liver-function tests and blood components may be affected by estrogen-containing OC's:
a. Increased sulfobromophination retention.
b. Increased prothrombin and factors VII. VIII, IX, and X, decreased antithrombin 3, increased norepinephrine-induced platelet aggregability.
c. Increased thyroid-binding clobulin (TBG) leading to increased circulating total-thyroid horizone, as measured by protein-bound iodine (PBI). T4 by column, or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG, free T4 concentration is unattered of. Decreased pregnancidol excretion.
e. Reduced response to metyrapone test.

Drug Interactions—Reduced efficacy and increased incidence of breakthrough bleeding have been associated with concomitant use of infampin A similar association has been suggested with barbiturates, phenyibutazone phenytoin sodium, ampucilian and tetracycline.

Carchogenesis, Mutagenesis, Impairment of Fertility—See Warnings section #3, 4, and 5 for information on carcinogenesis, mutagenesis, and impairment of fertility.

section #3, 4, and 5 for information on carcinogenesis, mulagenesis, and impairment of fertility.

Pregnacy—Category X. See Contraindications, Warnings.

Bursing Mothers — See Warnings. Because of the potential for adverse reactions in nursing infants from oral contraceptive tablets, a decision should be made whether to discontinue the drug, taking into account the importance of the drug to the mother.

Adverse Reactions—An increased risk of these serious adverse reactions has been associated with use of OC's (see Warnings) thrombophilebitis, pulmonary embolism, coronary thrombosis, cerebral thrombosis, cerebral thrombosis, cerebral hemorrhage, hypertension, galibilader disease, beingin hepatomas, congenital anomalies. There is evidence of an association between the following conditions and use of OC's afflowing adverse reactions have been reported in patients on OC's.

The following adverse reactions have been reported in patients on OC's The following adverse reactions have been reported in patients on OC's and are believed to be drug-related. Nausea and/or vomiting, usually the most common adverse reactions, occur in approximately 10 percent or less of patients during the first cycle. Other reactions, as a general rule, are seen much less frequently or only occasionally. Gastrointestinal symptoms (such as abdomina cramps and bloating), breakthrough bleeding, spotting, change in menstrual flow; dysmenorrhea, amenorrhea during and after treatment, temporary infertifity after discontinuance of treatment; edema; chloasma or melasma which may persist, breast changes; tenderness, enlargement, and secretion; change in weight (increase or decrease); change in cervical erosion and cervical secretion; possible diminution in lactation when given immediately postpartum; cholestatic jaundice, migrane, increase in size of uterine leiomyomata; rash (allergic); mental depression; reduced tolerance to carbohydrates; vaginal candidass; change in corneal curvature (steepening), intolerance to contact lenses.

The following adverse reactions have been reported in users of OC's, and the association has been neither confirmed nor refuted; premenstrual-like syndrome, cataracts, changes in libido, chorea, changes in appetite, cystins-like syndrome, headache, nervousness, dizzness, hirsufism, loss of scalp hair, erythema multiforme, erythema nodosum, hemorrhagic erup vaginitis, porphyria.

Serious ill effects have not been reported following Acute Overdose -acute ingestion of large doses of OC's by young children. Overdosage may cause nausea, and withdrawal bleeding may occur in females.



each tablet contains 0.15 mg levonorgestrel and 0.03 mg ethinyl estradiol

(LEVONORGESTREL AND ETHINYL ESTRADIOL TABLETS)



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CLINICAL SECTION

Clinical Opinion

Twin delivery: How should the second twin be delivered?

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In a series of 803 pairs of twins born between 1973 and 1982, 0.33% of second twins were delivered by cesarean section after vaginal delivery of the first twin. During the last year the frequency has increased to 7%, calling attention to the problem of declining obstetric skills and experience. This has caused us to update the routines of intrapartum management of twin gestations. In the present program only commonly available obstetric techniques are used. The potentially hazardous twin delivery is excluded from a trial of vaginal delivery. Hopefully, the program will help other obstetricians to decide in favor of vaginal delivery in selected twin gestations. (AM J OBSTET GYNECCL 1985;153:479-81.)

Key words: Twin gestation, delivery, cesarean section, ultrasound

It is hoped that articles on vaginal delivery in twin gestation, recently published by Chervenak et al.^{1,2} and Acker et al.,³ and the subsequent discussion,^{4,5} will encourage obstetricians to change their attitude from the policy of considering cesarean section as the optimal mode for delivery of twins. The consensus is in good agreement with our own opinion.

However, we have recently observed an increasing rate of cesarean section delivery of the second twin after vaginal birth of the first, which has caused us to update our routine for intrapartum management. In a series of 803 pairs of twins delivered during the period 1973 to 1982 in our county, the perinatal mortality was 3.6%. The cesarean section rate was 24%. Of those cases where the first twin was born vaginally, the second twin was delivered by cesarean section in only 0.33% of cases. During the recent 14 months, however, the incidence has increased to 7%, calling attention to the problem of declining obstetric skills and experience.

At the 1984 International Workshop on Twin Pregnancies we presented a paper⁶ regarding our current program for management of twin delivery. Our program is simple and only commonly available obstetric techniques are used; therefore, we think it worthy of being pre-

sented to the readers of the American Journal of Obstetrics and Gynecology.

Selection criteria

- 1. From the thirty-seventh week of gestation, vaginal delivery is planned, irrespective of the presentation of the second twin, if the first twin is in a vertex presentation
- 2. If the first twin is in a breech presentation after the thirty-sixth week, x-ray pelvimetry is performed. Critical measurements are 115 mm of the obstetric conjugate diameter and a sum of 325 mm of the anteroposterior diameter of the outlet, intertuberous diameter, and interspinous diameter, below which parameters a cesarean section is performed. Attention to the presentation of the second twin is so far insignificant. These criteria, when the first twin is in a breech presentation, are identical with our criteria for singleton breech delivery.
- 3. In the thirty-fourth to thirty-sixth gestational weeks, cesarean section is performed in cases of breech/breech, breech/vertex, and vertex/breech presentations. In cases of vertex/vertex and vertex/transverse presentations, vaginal delivery is primarily planned, unless other complications do not indicate cesarean section.
- 4. Before the thirty-fourth week, cesarean section is invariably performed, irrespective of the fetal presentations.

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Reprint requests: Per Olofsson, M.D., Department of Obstetrics and Gynecology, University Hospital, S-221 85 Lund, Sweden. 5. Cesarean section indicated by other complications, mainly growth retardation, is considered at the grounds of common obstetric indications and the because of the twin pregnancy per se.

Intrapartum management

- 1. The obstetrician leads the delivery from a position beside the abdomen of the parturient. This position is the most important, and the midwife is the delivery to make the vaginal examinations and manipulations primarily.
 - 2. An intravenous route is established.
- 3. Oxytocin and a rapid-acting tocolytic drug are prepared for immediate intravenous administration. We use terbutaline for tocolysis, given in single zoses of 0.25 to 0.5 mg.
- 4. The fetal heart rate of both twins is continue usly and simultaneously recorded; the fetal heart rate with first twin is preferably recorded by a scalp electronic and that of the second twin by an external ultrastand transducer. Uterine contractions are recorded by an external tocodynamometer.
- 5. An easily mobile real-time ultrasound appa = 1.s is available in the delivery room. Fetal positions and presentations have frequently already been determined during the first stage of labor.
- 6. During descent of the first twin, the second win is brought into a longitudinal position by externa renipulations under ultrasound assistance. Since this guiding is started before the birth of the first twin, he risk of transverse lie of the second twin is minimized. A vertex presentation of the second twin is optomal, but it is justified to deliver the twin in a breech presentation if necessary.
- 7. After the birth of the first twin, the oxymen infusion is immediately stopped if in use, and the end ond twin is firmly kept in a longitudinal position. The presentation is controlled by ultrasonic examination.
- 8. The leading part is then pressed down into the pelvic inlet and cervix by moderate pressure on the fundus. This means that we do not await spontaneous descent.
- 9. The midwife checks that the presenting part s well down in the cervix. In the case of uterine inelathe oxytocin infusion is then started slowly.
- 10. The membranes are ruptured during a uterize contraction. These maneuvers minimize the risk. I umbilical cord prolapse and cervical spasm.
- 11. A scalp electrode is applied. As long as the q____ity of the fetal heart rate record is good and no s g so of impending intrauterine asphyxia appear, there is absolute time limit for the interval elapsing between the births of the two babies.
- 12. If the external manipulations fail and the $\sec \propto d$ twin is in a transverse position, 0.25 to 0.5 mg of \implies

butaline is immediately given intravenously and the fetus is brought into a longitudinal position by external version under ultrasound control.

13. In the case of an ominous fetal heart rate pattern, the manipulations are of course quickened. If the external version is not successful, internal version and extraction are performed, with simultaneous suprapulic pressure on the fetal head.

Cesarean section has been recommended in twin pregnancies as the optimal mode of delivery in all presentations other than vertex/vertex, mainly in order to avoid a hazardous breech extraction of the second twin. Furthermore, the dangerous incidence of a transverse lie of the second twin after delivery of the first twin can be avoided. Routine cesarean section for vertex/breech and vertex/transverse positions has recently been questioned, however, and vaginal delivery of the breech second twin might be an alternative if the fetal weight exceeds 1500 gm (3½ pounds). On the other hand, the possibility of vaginal delivery of the breech first twin is hardly ever discussed.

In our present program, vaginal delivery is proposed in term twin gestations, irrespective of fetal presentations, provided the pelvis is not contracted. In preterm twin gestations we advocate cesarean section in the case of breech presentation of the first twin. This concept is based on experience from singleton preterm deliveries showing impaired outcome in breech presentations.9 In weeks 34 to 36 the management may be individualized. Experience is still too sparse for definite conclusions. Preliminary results from a Swedish national study (7714 twin pregnancies), from 1973 to 1981, indicate that an increasing cesarean section rate from 9% to 42% in gestational weeks 34 to 37 was not accompanied by a substantially reduced perinatal mortality (H. Rydhström, M.D., unpublished data). Until we have gained more experience, we believe it is wise to allow only vertex/vertex and vertex/transverse presentations to proceed to vaginal delivery in moderately preterm twin gestations. Before the thirty-fourth week, cesarean section should be performed by routine.

We respect the hesitation and cautiousness of American obstetricians, dealing with a more litigious society than we are used to. In our program, however, the potentially hazardous twin delivery is excluded from a trial of vaginal delivery. In general, ample time is available for manipulations during the second stage of labor. It is then important to perform the manipulations in the correct order, avoiding complications that would necessitate cesarean section for delivery of the second twin. Like the other debaters, 4.5 we have found intrapartum real-time sonography to be of great value. It enables direct visualization not only of fetal position but also of the beating fetal heart. With an attitude toward action, the time interval between the babies has

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no association with the outcome. I, ID, II There are no differences in mortality or early morbidity in the two babies even if the time interval exceeds 30 minutes.11, 12

Limited clinical experience of vaginal twin delivery is a growing problem.4, 5, 11 Ravburn et al.11 found a vaginal-abdominal delivery rate of 9%, which for them was surprisingly high but is on the same level as we recently experienced. During the past decade, the number of staff members taking an active part in obstetrics has doubled in many clinics. However, during the same period, the cesarean rate in twin gestations has more than doubled, thus leaving a diminishing number of vaginal deliveries for each obstetrician to experience.

Hopefully, the present program will help other colleagues to decide in favor of vaginal delivery in selected twin gestations.

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Clinical Articles

The effect of acute exercise on pulsatile release of luteinizing hormone in women runners

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Endurance exercise has been associated with reproductive dysfunction. We have previously suggested that pulsatile release of luteinizing hormone is impaired at rest in normal menstruating runners compared with sedentary women. To determine whether acute excess had any effect on pulsatile release of luteinizing hormone we investigated serum luteinizing connone levels in six normal menstruating runners at rest and after 60 minutes of running exercise. Exercise induced an increment in circulating luteinizing hormone levels greater than the change in hematocrit. We luteinizing hormone pulse frequency, calculated as the number of luteinizing hormone pulses per 6 hours, was reduced after exercise compared with values obtained at rest. There was no significant difference in pulse amplitude or area under the 6-hour curve between resting and postexercise situations. These data suggest that acute exercise has an inhibitory effect on luteinizing hormone pulsatile release 1 the hypothalamic level in eumenorrheic runners that is in addition to the previously described effect of sing. (AM J OBSTET GYNECOL 1985;153:482-5.)

Key words: Luteinizing hormone pulsatile releze, acute exercise, women runners

Intense physical exercise, particularly endurance training, has been associated with reproductive sovsfunction in women' and more recently in men.² = osssectional studies have suggested that 6% to 155 of women who are recreational runners and up to 5 ₹ of women who are competitive runners training abc ±30 miles per week may be amenorrheic.¹ Prospectiv∈ zudies have recently examined the effects of increang training on normally menstruating women rumaers preparing for a marathon. The investigations de not substantiate the previous finding that increasing mileage resulted in an increasing frequency of ameno 12 ea but reported that a majority of cycles in such wacen may be anovulatory or have an inadequate luteal pass.3 Decreased serum estradiol levels and impaired ge =d-. otropin response to gonadotropin-releasing horance were observed with increasing training mileage.4.2

The initiation of the menstrual cycle is deper ant

upon the pulsatile release of gonadotropin-releasing hormone, usually demonstrable as an increment in luteinizing hormone (LH) but not follicle-stimulating hormone levels occurring at approximately 90- to 120minute intervals in the early follicular phase of the menstrual cycle.7.8 Abnormalities of the LH pulse pattern have been associated with defects of the menstrual cycle, including hypothalamic amenorrhea,9 polycystic ovarian syndrome,10 and inadequate luteal phase.11 We have recently reported that LH pulse amplitude and frequency as well as the area under a 6-hour curve were decreased in a group of eumenorrheic runners compared with values in sedentary women.12 These data suggested that endurance training could exert a significant inhibitory effect on the hypothalamic-pituitary axis regulating the ovary. Such inhibition of the gonadotropin-releasing hormone-gonadotropin axis could be responsible for the primary and secondary amenorrhea that occurs in susceptible women. We wished to establish whether strenuous acute exercise had an inhibitory effect on LH pulsatile release; therefore, we compared the LH pulse patterns in six female runners at rest and after exercise.

Subjects and methods

Six runners training at least 32 km per week were accepted for the study after informed consent had been obtained. All subjects had menstrual cycles within the

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normal range and none had taken hormonal medication within the last 6 months. The study was approved by an ethics committee of the University of Alberta Hospital. The runners ranged in age from 20 to 30 years (mean 25.0 ± 1.5 years). All studies were carried out in the early follicular phase of the menstrual cycle (days 3 to 6). Height and weight of all subjects were recorded. Body fat was measured by a hydrostatic method according to the formula of Brozek et al. 13 and a maximal oxygen consumption test 14 was done in each subject. No subject had exercised in the 24 hours prior to an investigation.

Frequent blood sampling was performed through an indwelling intravenous cannula inserted into a suitable forearm vein 60 minutes before the beginning of sampling. Each subject underwent two studies carried out in successive menstrual cycles in random order beginning in the afternoon. In one study, the subjects had blood samples taken at 15-minute intervals for 6 hours as previously described.12 In the other study, blood samples were obtained at 15-minute intervals for 30 minutes before, 60 minutes during, and 6 hours following a 60-minute run at 11.2 km per hour on a treadmill with a slope calculated to be equivalent to 60% of maximal oxygen consumption. The blood samples were allowed to clot at room temperature, separated by centrifugation, and frozen at -20° C until assayed. Hematocrit was measured in heparinized Microcrit tubes during the exercise study in all samples. LH was measured in all samples by means of a commercially available radioimmunoassay (Immunonuclear Corporation, Stillwater, Minnesota) as described previously.12 The interassay variability was less than 10%. The intra-assay variability varied inversely with LH concentration, and for concentrations of 2.5, 5, 10, and 20 mIU/ml it averaged 20%, 10.5%, 7.5%, and 5% respectively. All samples from the same subject were assayed in a single assay. As a baseline, estradiol was measured in the first two samples of each study, also with a commercially available radioimmunoassay (Pantex, Santa Monica, California). In the case of estradiol, all samples were assayed together in a single assay with an intra-assay coefficient less than 10%.

LH pulses were identified in the 6-hour study without exercise and in the 6 hours after the 60-minute run in a manner similar to that previously described by Cumming et al.¹² and Reame et al.¹⁵ A pulse was determined as exceeding a significant increment, which was defined as a difference between the means of replicate determinations within 45 minutes that exceeded the minimum detectable increment. This value was calculated as the mean LH value multiplied by twice the intraassay coefficient of variation of replicate samples from the individual subject. The pulse frequency (number of pulses per 6 hours) was also calculated for the subject

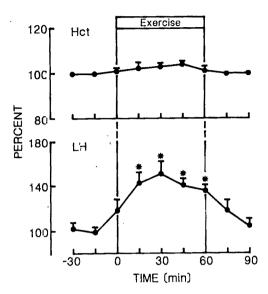


Fig. 1. Changes in serum LH levels and hematocrit measured 30 minutes before, during, and 30 minutes after 60 minutes' running exercise at 11.2 km per hour on a treadmill with a slope calculated to be equivalent to 60% of maximal oxygen consumption. The data are expressed as the percentage of change from baseline (mean of the first two samples). Mean baseline serum LH level was 6.8 ± 1.8 mIU/ml and the mean baseline hematocrit was $38\% \pm 1\%$. * = p < 0.05 compared with baseline value, Tukey test.

during each 6-hour period. Pulse amplitude was defined as the increase from the baseline value immediately preceding a pulse to the zenith of the pulse within 45 minutes. The area under each 6-hour curve was calculated by curve fitting according to Simpson's rule. Differences in LH pulse frequency (number of pulses per 6 hcurs), pulse amplitude, area under the LH curve, and baseline estradiol levels were evaluated by Student's paired t test.

The acute exercise-induced changes in LH and hematocrit were evaluated separately by two-way analysis of variance after transformation of the data to the percentage of change from baseline (the first two samples) to minimize the effect of individual variation in absolute values. Changes in individual values from baseline were evaluated with a post hoc Tukey test. Differences in responses of LH and hematocrit at specific time intervals were compared by means of Student's paired *t* test. The area under each 6-hour LH curve was calculated by curve fitting according to Simpson's rule.

Results

The mean height and weight of the runners were respectively 161.6 ± 2.2 cm and 54 ± 2.6 kg. The mean amount of body fat was $20\% \pm 1\%$ and the mean maximal oxygen consumption was 54.2 ± 3.5 ml/kg/min. All subjects accomplished the 11.2 km treadmill run.

During exercise, there was a significant increase in

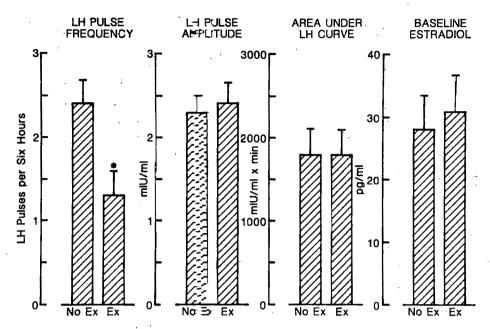


Fig. 2. LH pulse frequency, LH pulse and itude, area under LH curve, and baseline estradiol levels in six women runners following 24 heres without exercise (No Ex) and immediately after a 60-minute run at 11.2 km per hour (Ex). = p < 0.05, paired t test.

hematocrit and LH (Fig. 1, both p < 0.05, analys of variance). The increase in LH significantly exceed that of hematocrit at all points (p < 0.95, paired . Est). There was no significant increase in hematocrit at any specific time point (p > 0.05, Tukey test). LH sels began to increase before exercise in five of the six runners although values were not significantly different from baseline until 15 minutes after the start of the exercise (p < 0.05, Tukey test, Fig. 1). Levels remained elevated throughout exercise and dropped to base fire within 30 minutes after exercise.

LH pulse frequency was significantly lower after exercise than during the control period (Fig. 2, p < 1.05, paired t test). However, pulse amplitude and the area under the LH curve after exercise were not significantly different from values during the control nonexercise period.

Comment

Previous investigations of the effects of acute exercise on serum LH levels have produced contradictor—results. Bonen et al. 16 used running at approximately 7.5% of maximal oxygen consumption for 30 minutes at the exercise load and found no change in LH level in response to this exercise load. Jurkowski et al. 17 well-uated three consecutive exercise loads and, although there was a progressive increase with activity, the recrements were not significant by the statistical metleds used. LH levels were reported to be increased after a 10-mile race 18 and decreased after a competitive race athon run. 19 It is not possible to formulate an expanding at a progressive increased after a competitive race athon run. 19 It is not possible to formulate an expanding the statistical metled to the stat

nation for the discrepant results in the various studies. The consistency of the increase in LH levels in all subjects in the present study documented over several samples is impressive and the data are in keeping with the findings of Baker et al. 18 over a comparable distance.

To our knowledge, no investigations of pulsatile LH release after exercise have been published. We have shown previously that LH pulse frequency and amplitude as well as area under the LH curve were lower in eumenorrheic runners than in sedentary women. ¹² The results of the present study suggest that acute physical activity has a further inhibitory effect on pulsatile LH release in normally menstruating runners during the early follicular phase of the cycle. There does not appear to be any inhibitory effect on the pulse amplitude, and the area under the LH curve is virtually identical in postexercise and resting situations. However, the number of pulses per 6-hour period was significantly lower after exercise when compared with values obtained at rest.

Pulse amplitude may be modulated by circulating steroid hormones acting at the pituitary level. Pulse frequency may be changed by circulating steroids but the prime determining factor early in the cycle is the spontaneous activity of the hypothalamus. Therefore, alterations in pulse frequency at this time of the cycle suggest that acute exercise and training have an inhibitory influence at the hypothalamic level. We have previously postulated that exercise-associated amenorrhea results from the complex interaction of several factors together with a direct inhibitory effect of the physical

activity.20 The present data support that concept, but the complexity of training- and exercise-associated hormonal responses makes it impossible at this time to identify a neurotransmitter mechanism responsible for the changes.

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Postpartum disappearance of chorionic gonadotropin from the maternal and neonatal circulations

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The disappearance of chorionic gonadotropin from the $\pm i$ -culation was studied in a group of 10 healthy mothers and their offspring following vaginal delivery. Exelic analysis revealed the following biexponential clearance characteristics: in mothers the rapid half-life $\pm i$ -ponent averaged 4.75 \pm 0.58 (SE) hours (n = 6), and the slow half-life component averaged 32 \pm 1.35 hours (n = 6); in neonates the respective overall means were 1.32 and 55.2 hours. Total eliminal of chorionic gonadotropin (<0.005 |U/ml) occurred at a median time of 14 days following birth (renger 8 to 24 days) in mothers and at 1.5 days (range, 0 to 4 days) in neonates. (AM J OBSTET GYNECC_ 1585;153:486-9.)

Key words: Puerperium, neonate, chorionic goz azotropin, clearance

Notwithstanding the availability for over a decede of sufficiently sensitive and specific techniques for the measurement of chorionic gonadotropin in body Lods, few studies have appeared which examine the compearance of this hormone following normal vaginal delivery in either the mother or her neonate. We well to report our findings in 10 mothers and their offspring.

Material and methods

Ten healthy mothers and their infants were included for study on the basis of having undergone normaliserm gestation and vaginal delivery. The birth weights cline infants, six females and four males, ranged from £230 to 3970 gm; all had Apgar scores of 9 or 10 a new minute following delivery. Written informed consent was obtained from the mothers. The study wa approved by the Faculty Committee on the Use of Huran Subjects in Research.

Peripheral blood samples were obtained from he mothers during labor (usually at the time of del rery of the placenta), at 2- to 12-hour intervals during the first day, daily for the next 4 to 5 days, and weekly thereafter for 3 to 4 weeks. Infant blood samples were collected by heel prick at birth, at 6- to 12-hour intervals during the first day and daily for the following 3 to 5 days. In addition, umbilical cord blood samples were obtained from a group of 30 normal newborn infants.

Serum samples were kept frozen (-20° C) until assayed.

All maternal, infant, and cord blood samples were analyzed for chorionic gonadotropin by a β-subunit assay in which physiologic levels of pituitary luteinizing hormone are not recognized.1.2 Values are expressed as international units in terms of the Second International Standard for human chorionic gonadotropin. All samples from an individual subject were assayed in the same assay run. Selected maternal samples from day 3 on were also analyzed in an α-subunit assay with similar characteristics to that of the method of Kourides et al.,3 but in which the cross-reactivity by intact purified chorionic gonadotropin was approximately 1.5% on a weight basis. The assay reagents employed were as follows: (1) an antihuman luteinizing hormone α -subunit rabbit antiserum No. 1 provided as a gift from the National Pituitary Agency, National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases, and used at an initial dilution of 1:8000; (2) α-subunit of human chorionic gonadotropin (CR 119α) prepared by Dr. Robert E. Canfield, received as a gift of the Center for Population Research of the National Institute of Child Health and Human Development, and used as the radioligand (iodinated with iodine 131 by the chloramine T method⁴) as well as the assay standard. The minimum detectable levels were 5 mIU/ml (0.1 ml sample) in the β-subunit of chorionic gonadotropin assay and 0.1 ng/ml (0.1 ml sample) in the α-subunit assay. Coefficients of intra-assay variation ranged from 5% to 10% over the entire assay range in both assays.

Calculation of chorionic gonadotropin decay was performed with use of a biexponential computer program developed by Dr. F. S. Chebib, University of Manitoba, Faculty of Dentistry. This method, based on that

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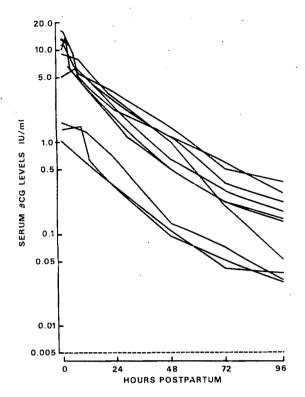


Fig. 1. Disappearance of chorionic gonadotropin (CG) from the maternal circulation following delivery. Note the logarithmic ordinate scale. The dashed line indicates the limit of assay detectability.

of Hartley,5 uses an iterative least squares approach and provides an estimate of goodness of fit (r value). The overall formula to fit the data is in the form $C = Ae^{-\alpha t} + Be^{-\beta t}$, where C is the serum concentration, t is the time, A and a are the fast component parameters (y intercept and slope, or fractional rate of disappearance, respectively), and B and β are the slow component parameters. Calculation of half-life was performed according to the following formula: $t_{1/2}$ = 1n2/slope (1n2 = 0.6933; slope is α or β).

Results

Maternal serum chorionic gonadotropin levels at delivery ranged from 1.3 to 16.0 IU/ml whereas the concentration in their newborn infants was < 0.005 to 0.35 IU/ml. The median umbilical cord serum chorionic gonadotropin level in the additional group of 30 newborn infants was 0.028 IU/ml (range: <0.005 to 4.78 IU/ml).

The disappearance of chorionic gonadotropin was rapid in mothers during the first 8 to 24 hours after delivery (Fig. 1 and Table I). In only six of the 10 mothers could statistically significant disappearance kinetics be calculated. Of the four subjects in whom analysis failed to reach statistical significance, two showed an increment in postpartum chorionic gonadotropin levels of 22% and 7% at 7 and 9 hours, respectively

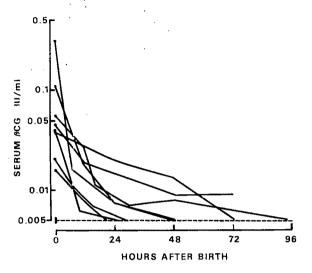


Fig. 2. Disappearance of chorionic gonadotropin from the neonatal circulation following delivery. Note the logarithmic ordinate scale. The dashed line indicates the limit of assay detectability.

(most likely due to retained functioning placenta although no confirmatory α-subunit determinations were made-see below), and the other two had had insufficient data collected. The rapid disappearance component averaged 4.75 hours (range, 2.9 to 6.7 hours). This was followed by a slow disappearance component that averaged 32.22 hours (range, 26.1 to 34.7 hours).

To rule out the possibility that the slow decay component, at least in mothers, was not a result of retained viable placental tissue, we measured the α-subunit of chorionic gonadotropin in selected maternal samples on and after postpartum day 3. Since free α-subunit is secreted during normal pregnancy,6 but the decay of this subunit is rapid (fast half-life component approximates 13 minutes; slow half-life component approximates 76 minutes⁷), we reasoned that the finding of αsubunit ir excess of the known cross-reactivity by intact chorionic gonadotropin in the α-assay (approximately 1.5%) would indicate retained functioning placental tissue. In fact, no free α -subunit activity was detected in any of the selected maternal samples assayed.

Insufficient data collection precluded detailed analyses of individual neonates. Overall, two disappearance components were observed (Table I and Fig. 2). The fast component ($t_{1/2} = 1.32$ hours) was considerably more rapid than that of the mothers whereas the slow component ($t_{1/2} = 55.23$ hours) was considerably more prolonged than that of the comparable maternal component.

Total elimination of chorionic gonadotropin (<0.005 IU/ml) occurred at a median time of 14 days following birth (range, 8 to 24 days) in mothers and at 1.5 days (range, 0 to 4 days) in neonates (Table II).

Table I. Kinetic parameters for chorionic gonadaropin disappearance from the maternal and neonatal circulations following delivery*

Group	Subject	Initial chorio rz gonadotropin v. 22 (IU/ml)	Rapid component half-life (hr)	Slow component half-life (hr)	r value	p value
Maternal	А. J.	16.0	3.7	34.7	0.993	< 0.01
	M. L.	13.1	5.9	31.3	0.993	< 0.01
	E. W.	13.0	4.2	34.5	0.942	< 0.01
	L. R.	11.2	5.1	26.1	0.998	< 0.01
	M. M.†	10.0	2.9	34.4	0.820	< 0.01
	R. K.	9.2	6.7	32.3	0.718	< 0.05
	Overall mean	12.08	4.75	32.22		
	SD ·	2.48	1.42	3.30		
	SEM	1.01	0.58	1.35		
Neonatal	All infants	0.084	1.32	55.23	0.547	< 0.01

^{*}See Material and methods for statistical analysis.

Table II. Estimated time for total elimination of chorionic gonadotropin from the maternal and neonatal circulations following delivery*

	Days to total disappearana of choricnic gonadotropiz				
Subjec!	Maternal	Neona=l			
A. J.	14	. 2			
M. L.	14	2 3			
E. W.	24†	4÷			
L. R.	22	2			
M. M.	· 14	I			
R. K.	. 14	I			
S. S.	14	ī			
B. S.	8	0‡			
S. P.	22†	4†			
R. W.	8	0‡			
Overall median	14	1.5			
Range	8-24	0-4			

^{*}Chorionic gonadotropin no longer detected ($<0.0 \ 5 \ IU/ml$) on the day indicated.

Comment

Surprisingly, little information is available regarding the kinetics of chorionic gonadotropin disappea and in either mother or child following delivery. Material studies have been confined to a small number of women. Furthermore, these previous studies i many porated an infrequent sampling regimen, and in addition, data from a total of only 16 normal variable deliveries were collected. Despite these limitations, the disappearance of chorionic gonadotropin could be described by both bioassays and radioimmuno stay methods by a biexponential function with interial rapid, and slow half-life characteristics not substantial.

different from the means near 5 hours and 32 hours, respectively, which we obtained.

On theoretical grounds as well as from observations we made in three subjects in whom chorionic gonadotropin values were higher in the first 2 to 9 hours following delivery than at delivery, residual trophoblastic tissue may complicate the analysis. Indeed, we were unable to derive any meaningful kinetic data from two of these three subjects. Nonetheless, the kinetic values we obtained are remarkably similar to those observed following the administration of exogenous chorionic gonadotropin to nonpregnant subjects. For example, average values for the rapid and slow component of approximately 6 and 36 hours, respectively, following the acute intravenous bolus administration of purified chorionic gonadotropin were obtained recently, 13 values not dissimilar to ones obtained following the administration of relatively impure chorionic gonadotropin preparations.14.15

Although after the first week our sampling regimen was infrequent, we estimated that complete disappearance of chorionic gonadotropin (<0.005 IU/ml) occurred by a median time of 14 days (range 8 to 24 days) in our 10 subjects. A previous study with use of identical assay methodology reported chorionic gonadotropin disappearance at 11 and 16 days in two subjects following term pregnancy.16 On theoretic grounds, the time to chorionic gonadotropin elimination should reflect initial level, the presence of retained functioning placental tissue, and the assay sensitivity (without luteinizing hormone cross-reactivity). Not surprisingly, therefore, total elimination of chorionic gonadotropin has been reported to average 38 days (range, 29 to 44 days)17, 18 following first-trimester suction curettage in patients in whom initial serum chorionic gonadotropin levels were considerably higher than the ones we encountered at term; an additional factor as well may be

[†]Subject in whom value at 2 hours after delivery (45 IU/ml) exceeded that at delivery.

[†]Estimated value based on last detectable value and regal mean slow component for disappearance for appropriate group (see Table I).

[‡]Undetectable value (<0.005 IU/ml) at birth.

retained products of conception, which have also been reported for patients with ectopic pregnancy.19

Kinetic analysis of chorionic gonadotropin disappearance from serum with use of pooled data from neonates also revealed a biexponential rate of removal. The fast and slow components in neonates differed from those in mothers, being more rapid initially (mean of 1.3 hours compared to 4.8 ± 0.6 hours) and less rapid subsequently (mean of 55 hours compared to 32 ± 1.4 hours). No reliable quantitative kinetic data have been published previously for neonates, although biexponential disappearance has been suggested¹² and an approximate initial half-life of 2 hours can be calculated from 2 to 6 hours following birth from the data of Cacciari et al.26 The time for total chorionic gonadotropin elimination (<0.005 IU/ml) by the neonate which we observed, median 1.5 days (range, 0 to 4 days), is similar to that suggested by previously published data.21.22 The explanation for the difference in kinetic parameters between mother and child is unclear; certainly the more rapid early disappearance component from serum in infants compared to mothers cannot be explained by the apparent less rapid fractional urinary clearance in neonates21 than in adults.8.13 Thus nonrenal clearance factors must be operative here.

In summary, we have described biexponential disappearance characteristics to the clearance of chorionic gonadotropin from the circulation in a group of 10 mothers and infants following vaginal delivery. Total elimination of chorionic gonadotropin (<0.005 IU/ml) occurs at a median time of 14 days (range, 8 to 24 days) in mothers and at 1.5 days (range, 0 to 4 days) in neonates following birth.

We wish to thank Dr. F. S. Chebib for statistical advice and Miss J. Clements for technical assistance.

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Interpretation of nonstress tests

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Five nonstress tests were interpreted by a national sample of obstetricians blinded to specific patient clinical data. The 169 questionnaires suitable for analysis €howed that the reliability of the test interpretation, as measured by the kappa statistic, decreased with increasing number of categories of interpretation. Kappa values for two, three, and five categories of interpretation were 0.60, 0.39, and 0.36, respectively. The middle values in the three- and five-category methods of interpretation had very low levels of reliability. Kappa values as related to the age of the respondent or measurements of experience showed relatively small changes in reliability of interpretation. (AM J OBSTET GYNECOL 1985;153:490-5.)

Key words: Nonstress test, interpretation, reliabil.

During the last 10 years the nonstress test has become a primary method of antepartum fetal testing in the United States.1 Despite its popularity, various autrors have described relatively low reliability of nonstress test interpretation.2-4 Flynn et al.5 noted that different methods of reporting the test resulted in different interobserver variability rates. In addition, Bobitt⁶ descr. 52d five cases in which errors of interpretation resulter in the deaths of infants. He felt that the errors resulted in part from a lack of experience with the nonstress test. This study was designed to test reliability of ane nonstress test interpretation with use of three different methods of reporting the interpretation. The reliability of test interpretation was also correlated with measurements of age of the respondent and nonstress test experience.

Methods

One and one-half years of files (January, 1983 to June, 1984) from the fetal diagnostic unit of Duke University Medical Center were reviewed to obtain 15 cz :es that represented the range of tests recorded during wis period. Representative 20-minute samples were reduced in size and reproduced to fit on legal-size papar. Of these 15, five were chosen after review by members of the Division of Maternal-Fetal Medicine for acceptaable quality of reproduction.

A pilot study with use of these five tests combination with demographic questions was carried out between November 15, 1984, and January 15, 1985. After xeview of the results of this study the questionnaire was

redesigned and mailed to a national sample of obstetricians obtained from the 1983-84 American College of Obstetricians and Gynecologists Directory of Fellows. The mailing was sent on February 15, 1985, and the study was closed on May 15, 1985. An additional encouragement letter was sent on March 15, 1985, to nonresponders. The study was conducted anonymously by use of a separate postcard return.

Responses were entered in a microcomputer database management program. These responses were subsequently transferred to the Triangle University Computation Center and analyzed with the SAS statistical analysis software.*

Results

Survey response. One thousand addresses were chosen from the 1983-84 Directory of Fellows. Of these, 858 were correct addresses; 183 questionnaires were returned for a response rate of 18.3%, and 169 questionnaires contained enough information for interobserver variability analysis. Table I describes the samples by age and Table II by American College of Obstetricians and Gynecologists district of the respondent. A significantly younger group of individuals responded to the questionnaire. There were decreased response rates from American College of Obstetricians and Gynecologists Districts 1 and 3. The postcard response was notable in that many of the nonrespondents were retired or no longer practicing obstetrics.

Fig. 1 illustrates the nonstress test results interpreted by the respondents. Table III lists the responses by method of interpretation and individual categories of response for each of the nonstress tests. The two-category method of interpretation asked the respondent to interpret the test as either "reactive" or "nonreactive." The three-category method of interpretation

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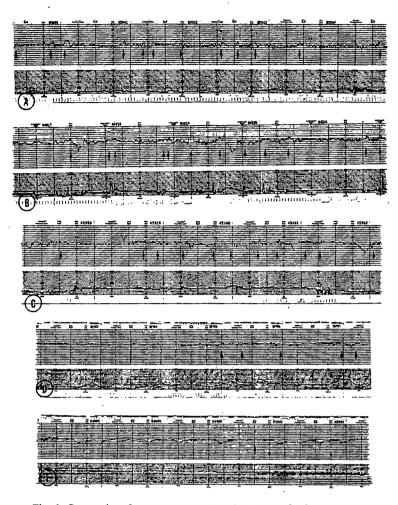


Fig. 1. Composite of nonstress tests A to E. Arrow = fetal movement.

Table I. Description of samples by age

	Original	sample	Returned sample		
Age (yr)	n	%	n	%	
<30	5	0.5	0		
30-39	272	27.2	65	39.63	
40-49	298	29.8	60	36.59	
50-59	213	21.3	32	19.51	
≥ 60	212	21.2	7	4.27	
Total	1000		164*		
Mean age	49.26 ±	13.175	43.42	± 8.137	

 $[\]chi^2$ test for heterogeneity, 4 degrees of freedom = 39,940, p << 0.001.

asked the respondent to interpret the test as "reactive," "equivocal," or "nonreactive." "Equivocal" was defined as "all other nonstress test abnormalities." In the fivecategory method of interpretation the respondent was asked to interpret the test by ranking the "health" of the fetus from "1" = most healthy to "5" = most ill. Overall, there was a good response rate by three different methods, although there was a significantly de-

Table II. Description of samples by geography

American College of Obstetricians and	Orig sam		Returned sample		
Gynecologists district	n	%	n	%	
1	34	3.4	3	1.69	
2	68	6.8	13	7.34	
3	82	8.2	8	4.52	
4	193	19.3	32	18.08	
4 5.	94	9.4	17	9.60	
ŝ	131	13.1	25	14.12	
7	129	12.9	22	12.43	
8	180	18.0	38	21.47	
9	89	8.9	19	10.73	
Total	1000		177*		

 $[\]chi^2$ test of heterogeneity, 8 degrees of freedom = 8.12, = 0.422.

creased response rate to the three-category method of interpretation. There were no significant differences in response rates to each of the tests.

Reliability and number of categories. Reliability is a measurement of agreement or reproducibility. A

^{*}Five ages were unknown.

^{*}Six zip codes were unknown.

Table III. Responses by method of interpretation and individual categories of response for each nonstress test (n = 169)

					Method of i	nterpretation				
		Two-ce	ategory				Three	e-category		
	Red	ıctive	Ņon	reactive	Rec	Reactive Equivocal		uivocal	Nonreactive	
Test	n	%	n	ι λ 0	n	%	n	%	n	%
A	159	97.55	4	۶4.	121	96.03	2	1.58	3	2.38
В	123	85.42	21	14.5≡	63	46.38	59	43.38	14	10.29
C	26	17.81	120	821	7	5.15	65	47.79	64	47.06
D	26	17.33	124	82-6	7	5.39	59	45.38	64	49.23
E	7	4.64	144	95.36	1	0.81	21	16.94	102	82.25
catego	l by numbe ories	r of	. 7	754			٠.		6	552
Catego Total re	ories esponses						•			

Percentage of possible responses = 85.44% (2166 \supset £ 2535). A two-way nonreplicated analysis of variance for categories and tests was calculated. Responses by categories, p = $0.\Box$ L Responses by tests, p = 0.86.

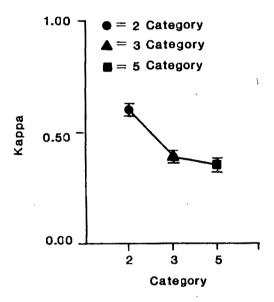
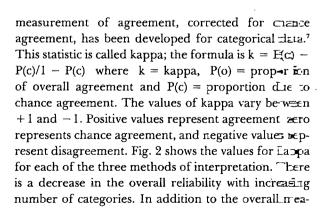


Fig. 2. Reliability and method of interpretation. Kappævelles for the three methods of interpretation are given. Two-category: number, 120; kappa, 0.60 ± 0.01. Three-caægery: number, 105; kappa, 0.39 ± 0.01. Five-category: number, 133; kappa, 0.35 ± 0.01.



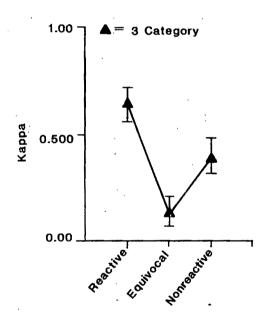


Fig. 3. Individual category reliabilities with three-category method of interpretation. Kappa values for reactive, equivocal, and nonreactive tests are 0.64 ± 0.06 , 0.13 ± 0.06 , and 0.39 ± 0.07 , respectively. Equivocal was defined as "all other nonstress test abnormalities."

sures of agreement, individual category kappas can be calculated for each response. Figs. 3 and 4 show the individual category kappa values. There is no statistical difference between the healthy categories in all three methods of interpretations. The middle values show the poorest reliability, with a decrease in reliability of the nonreactive category in the three-category method of interpretation.

Reliability and age of respondent. Fig. 5 shows the kappa values for each of the three methods of interpretation as related to the age of the respondents. The reliability of interpretation tended to increase with age

				Five	-category				
	1		2		3		4		5
n	%	n	%	n	%	n	%	п	%
135	88.76	18	11.69	1	0.65				
23	15.33	52	34.67	55	36.67	14	9.33	6	4.00
4	2.61	11	7.19	63	41.18	66	43.14	9	5.88
3	2.01	33	22.15	75	50.34	32	21.48	6	4.03
1	0.65	4	2.60	4	2.60	21	13.64	124	80.53
									760

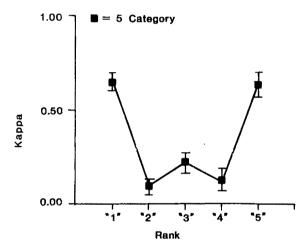


Fig. 4. Individual category reliabilities with five-category method of interpretation. Kappa values for interpretation by ranks. Ranks were defined as I = most healthy to 5 = most ill. Individual values for ranks are $I = 0.65 \pm 0.04$, $2 = 0.09 \pm 0.03$, $3 = 0.22 \pm 0.04$, $4 = 0.13 \pm 0.04$, and $5 = 0.64 \pm 0.04$.

for the two-category method of interpretation and decrease with the three- and five-category methods of interpretation. The number of respondents in the age group "equal to and over 60" used for these calculations were five, three, and two for the two-category, three-category, and the five-category interpretations, respectively. The numbers in the other age categories ranged from 16 to 53 with an average of 37.88 ± 14.21 .

Reliability and measures of nonstress test experience. Three measures of nonstress test experience were used: years of nonstress test use, number of patients tested per month, and respondent's self-reported level of experience. Figs. 6, 7, and 8 show the kappa values for these groups. The number of respondents used for the calculations was lowest in the category of 0 to 3 years of nonstress test use and decreased with number

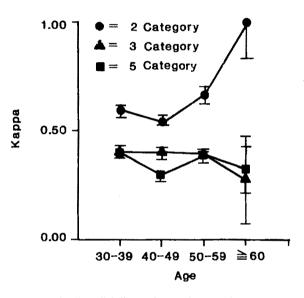


Fig. 5. Reliability and age of respondent.

of patients tested per month. There were three categories of self-reported experience: minimal, average, and a lot. Too few responded to the minimal category, and there were no significant differences between the other groups of self-reported experience. These data show no consistent trend in reliability with years of nonstress test use, no consistent trend in reliability with the number of patients tested per month, and only a slight improvement in the reliability of interpretation with increasing experience in the two- and five-category interpretations in the subgroup with "a lot" of experience. The changes are small in comparison to the larger effect of the number of categories.

Comment

Studies of reliability of interpretation of electronic fetal diagnostic tests have been carried out with the nonstress test, 2-5 with the oxytocin challenge test, 8 and

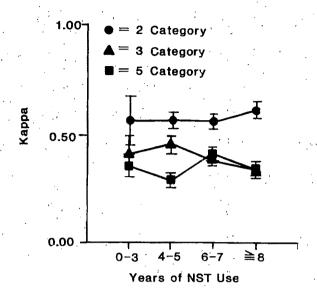


Fig. 6. Reliability and years of nonstress test us.

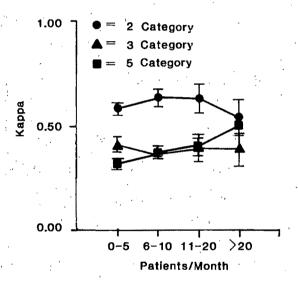


Fig. 7. Reliability and patients tested per month

with intrapartum electronic fetal monitoring. 4.9-15 T is study is the first to attempt to use survey methodology to study reliability in a larger sample of observer. With the nonstress test. The nonstress tests that were presented were limited in number but attempted to present the range of tests recorded. In addition, the tests oid not attempt to present to the observers any tests of poor quality or ones with arrythmias in the fetus that would lead to confusion. The lack of specific clinical data, particularly the gestational age, is unlike reality in that these data are usually available. Despite these deviations from the usual clinical situation, the following observations can be made about the reliability of interpretation of the nonstress test by this survey:

1. The reliability of interpretation of the nonerest test is only "fair" with the simplest method of classication and "low" with more complicated methods of classification of abnormalities of the test.

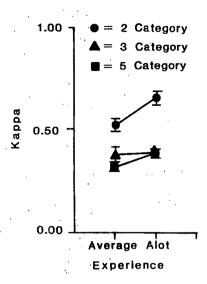


Fig. 8. Reliability and self-reported experience with the nonstress test.

- 2. The number of categories and the definitions of both normal and abnormal criteria for interpretation significantly influence reliability of this test. This lack of a standard classification seriously limits the reliability. Characteristics of a "gold standard" method of classification would be clarity of definitions with a limited number of classes.
- 3. The relatively small effects of either age or particular measures of experience with the nonstress test on reliability of interpretation by this sample was an unexpected finding. There is no easy explanation for this finding. However, other authors using "expert" observers have not shown high levels of reliability of interpretation.^{2,4}

It is important to realize that the reliability of this test is also a result of other factors related to the performance of the test, biologic variability, the equipment, and personnel used. This report did not address these issues.

This test is very popular and appears to hold promise in reducing perinatal morbidity and mortality. However, the reliability of test interpretation needs improvement. This improvement can best be accomplished by definition of a "gold standard" classification. This standard classification would also allow comparisons to be made between other studies of its use.

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Triphasic oral contraception: Metabolic effects in normal women and those with previous gestational diabetes

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The effect of a low-dose triphasic ora contraceptive (ethinyl estradiol and levonorgestrel) on glucose tolerance, plasma insulin and glucagon responses to glucose, fasting plasma cortisol, triglycerides, free fatty acids, total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, and very-low-density lipoprotein cholesterol was investigated in 16 women with previous gestational diabetes and in 19 normal women. Investigations were performed prior to the hormonal intake and after treatment for 2 and 6 months. Before treatment, the women with previous gestational diabetes had significantly elevated fasting glucose (p < 0.05) and impaired glucose tolerance (p < 0.05) when compared to those of the healthy control subjects. The glucose, insulin, and glucagon responses to oral glucose remained unchanged during the treatment period. Plasma cortisol increased in both groups (p < 0.05) whereas plasma triglycerides increased in the control subjects only (p < 0.05). Plasma ree fatty acids, lipoproteins, and high-density lipoprotein cholesterol/total cholesterol ratio remained unchanged in both groups. The results suggest that a low-dose triphasic oral contraceptive (ethinyl estradiol and levonorgestrel) is suitable as contraception even in women with a previous deterioration of glucose tolerance during pregnancy. (AM J OBSTET GYNECOL 1985;153:495-500.)

Key words: Triphasic oral contraceptive, gestational diabetes, glucose tolerance, cortisol, lipid metabolism

In 1963, Waine et al.¹ published the first report on alterations in carbohydrate metabolism following intake of oral contraceptives. Since then the risk of developing a deterioration of glucose tolerance during intake of the combined estrogen/progestogen compounds has been substantiated².³ and, together with the risk of consistent changes in plasma lipid levels,⁴ this has caused great concern. As a consequence new low-

dose formulations with, first and foremost, reduced estroger content but also containing new progestogens have been developed in the search for preparations with less potential risk.

Triphasic preparations of ethinyl estradiol and levonorgestrel with a progestogen content less than that in any of the monophasic products have been introduced recently. As the estrogen/progestogen ratio of the combined oral contraceptives seems to be of major importance for the metabolic effects, ^{5, 6} we were prompted to study the influence of a triphasic compound on glucose tolerance and plasma lipid/lipoprotein levels in women with a previously reduced glucose tolerance during pregnancy (gestational diabetes). For comparison a group of normal women was also investigated.

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Table I. Fasting values (mean \pm SEM) of plasma glucose, insulin, glucagon, and cortisol in women with previous gestational diabetes (Prev. GDM) (n = \oplus) and nondiabetic control subjects (n = 19) using a low-dose triphasic oral contraceptive*

	Pretreatment		2	mo	б то		
	Prev. GDM	Controls	Prev. GDM	Controls	Prev. GDM	Controls	
Glucose (mmol/L)† Insulin (pmol/L) Glucagon (pmol/L) Cortisol (nmol/L)	$5.4 \pm 0.1 \ddagger$ 95 ± 8 19 ± 2 452 ± 52	$4.9 \pm 0.$ $95 \pm 11.$ 21 ± 2 459 ± 33	5.3 ± 0.2 110 ± 12 17 ± 2 558 ± 39	5.1 ± 0.1 111 ± 9 20 ± 2 643 ± 25	$5.4 \pm 0.2 \ddagger$ 108 ± 9 15 ± 1 601 ± 46 §	5.0 ± 0.1 112 ± 9 20 ± 3 649 ± 27	

^{*}Oral contraceptive containing ethinyl estradiol and _emorgestrel.

Table II. Incremental glucose and insulin areas and decremental glucagon area (AUC) (mean \pm SEM) calculated from oral glucose tolerance tests perfected in women with previous gestational diabetes (Prev. GDM) (n = 16) and in nondiabetic control subject (n = 19) during intake of low-dose triphasic oral contraceptive*

	Pretreæn e e		2 1	no	6 mo	
	Prev. GDM	Controls	Prev. GDM	Controls	Prev. GDM	Controls
AUC glucose (mmol × min × L ⁻¹) AUC insulin (nmol × min × L ⁻¹) AUC glucagon (pmol × min × L ⁻¹)	259 ± 50† 36 ± 5 392 ± 93	IBI ± 20 虹 ± 7 5毫 ± 104	265 ± 46 39 ± 5 433 ± 100†	187 ± 29 36 ± 3 751 ± 133	299 ± 42† 38 ± 18 353 ± 84†	163 ± 34 38 ± 17 849 ± 174

^{*}Oral contraceptive containing ethinyl estradiol and exenorgestrel.

Material and methods

Subjects. Sixteen women with previous gestational diabetes and 19 nondiabetic women were recruited in to the study. Informed consent was obtained from all participants and the study was approved by the local e h.cs committee. All women were within 10% of their fileal body weight. The mean age of the women with previous gestational diabetes [28.7 ± 1.1 (SEM) years, rang= 17 to 30 years] was not significantly different from that of the controls [25.9 ± 0.1 (SEM) years, range 23 to 35 years]. All participants were at least 6 months post partum, not breast-feeding, and without hormonal treatment for 3 months before entering the study.

In the women with previous gestational diabetes the average period from the diagnostic oral glucose to-erance test was 9 months (range 6 to 18 months). Gestational diabetic patients had normal blood glucose concentrations in the fasting state but had diabetes daynosed for the first time after a 50 gm oral glucose tolerance test in pregnancy. In the women with gestational diabetes, at least two glucose values of the end glucose tolerance test exceeded the mean + 3 SD curre in a group of 46 normal nonpregnant control subjects evaluated by exactly the same procedure. Our days

nostic criteria correspond closely to those advocated by the National Diabetes Data Group.⁸

Treatment. The triphasic pills were administered cyclically in 3-week periods followed by one medication-free week for 6 months. In each period, 30 μg of ethinyl estradiol plus 50 μg of levonorgestrel were taken during the first 6 days, 40 μg of ethinyl estradiol plus 75 μg of levonorgestrel for the next 5 days, and 30 μg of ethinyl estradiol plus 125 μg of levonorgestrel during the last 10 days.

Investigative procedure. A 50 gm oral glucose tolerance test was performed in all women before treatment was started and after tablet intake for 2 and 6 months. Blood pressure and body weight were recorded at each testing. Before treatment, the oral glucose tolerance test was always performed in the secretory phase (days 21 to 28) of the menstrual cycle and during treatment, in the last phase of the tablet intake (days 15 to 21). All participants reported to the laboratory in the morning, after an overnight fast and abstinence from smoking and following at least 3 days of unrestricted diet (>150 gm of carbohydrate) and physical activity. A short plastic catheter was inserted into an antecubital vein and blood samples were drawn in

[†]To convert glucose concentration in millimoles per .ies to milligrams per deciliter multiply by 18.

[‡]Women with previous gestational diabetes versus control subjects: p < 0.05.

[§]Pretreatment versus 6 months: p < 0.05.

^{||}Pretreatment versus 2 months: p < 0.05.

[†]Women with previous gestational diabetes versus co_t-o subjects: p < 0.05.

chilled heparin-aprotinin tubes before the glucose challenge and 15, 30, 45, 60, 90, 120, 150, and 180 minutes after. Tubes were centrifuged at 4° C and plasma stored at -20° C until assayed.

Plasma glucose was determined by a dehydrogenase method.9 Plasma insulin, glucagon, and total cortisol were measured by previously described radioimmunoassays7, 10, 11 and a radiochemical assay was used for determination of free fatty acids.12 Determination of total cholesterol and triglyceride levels was performed enzymatically by means of commercially available kits (CHOD-PAP and GPO-PAP, Boehringer-Mannheim A.G., Mannheim, West Germany). High-density lipoprotein cholesterol was determined after removal of low-density lipoprotein cholesterol and very-low-density lipoprotein cholesterol by precipitation with phosphorus-tungsten acid-magnesium chloride. The level of very-low-density lipoprotein cholesterol was calculated by the formula of Friedwald et al.18 and the lowdensity lipoprotein cholesterol level was obtained by subtraction of high-density lipoprotein cholesterol and very-low-density lipoprotein cholesterol from total cholesterol values.

Calculations and statistics. A numerical expression for glucose tolerance was obtained by integration of each glucose concentration curve during 0 to 120 minutes with the fasting level used as a baseline, that is, the incremental area under the curve. The period 0 to 120 minutes was chosen to avoid the possible effect of the rebound hypoglycemia that was frequently found after this time. The incremental insulin areas and the decremental glucagon areas were calculated similarly. Results are presented as mean ± SEM. Comparison within one group of women was done by Student's t test for paired data. Comparisons between groups were estimated by Student's t test for unpaired data when variances of the groups were not significantly different judged by the variance ratio. When variances between groups were different, the Mann-Whitney U test was applied. Two-tailed p values < 0.05 were considered significant.

Body weight and blood pressure. No changes in body weight and blood pressure were found during the study.

Plasma glucose, insulin, glucagon, and cortisol

Fasting values (Table I). Before treatment fasting glucose values were significantly higher in the women with previous gestational diabetes than in the control subjects. This difference persisted after hormonal intake for 6 months. No changes in fasting glucose levels were observed within the groups during the study.

During treatment a slight but significant increase in

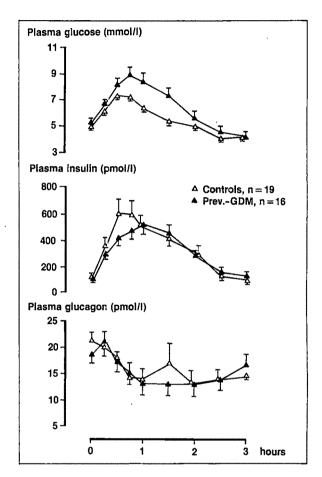


Fig. 1. Plasma glucose, insulin, and glucagon values (mean ± SEM) during oral glucose tolerance tests performed in women with previous gestational diabetes (Prev.-GDM) (n = 16) and in nondiabetic control subjects (n = 19) before intake of a lowdose triphasic oral contraceptive (ethinyl estradiol and levonorgestrel).

fasting insulin levels was observed in the control group. No changes occurred in the women with previous gestational ciabetes.

Fasting glucagon levels did not change in either group during the study.

Fasting total cortisol levels increased significantly and similarly in both groups during treatment.

Oral giucose telerance test. Before treatment plasma glucose values (Fig. 1) and the areas under the curves were higher in the women with previous gestational diabetes than in the control subjects (Table II). No changes in the plasma glucose values and the areas under the curves were observed within the groups during the study.

Although the insulin response to the glucose load appeared to be slightly blunted in the women with previous gestational diabetes at the start of the study (Fig. 1) the areas under the curves were not significantly different in the women with previous gestational dia-

Table III. Fasting values (mean \pm SEM) of plasme triglycerides, free fatty acids (FFA) and the high-density lipoprotein (HDL) cholesterol/total cholesterol ratio in women with previous gestational diabetes (Prev. GDM) (n = 16) and in nondiabetic control subjects (n = 19) using a low-dose triphasic oral contraceptive*

	Pretreatnæn:		2	то	6 mo	
	Prev. GDM	Car.trols	Prev. GDM	Controls	Prev. GDM	Controls
Triglyclerides (mmol/L) FFA (mmol/L) HDL cholesterol/total cholesterol ratio	1.04 ± 0.1 767 ± 58 0.26 ± 0.01	09 ± 0.06 69 ± 63 025 ± 0.01	1.01 ± 0.08 743 ± 41 0.28 ± 0.01	1.08 ± 0.09† 737 ± 67 0.29 ± 0.02	1.10 ± 0.09 738 ± 58 0.28 ± 0.01	$1.06 \pm 0.08 \ddagger$ 699 ± 56 0.31 ± 0.02

*Oral contraceptive containing ethinyl estradiol anc lezonorgestrel.

betes and the control subjects (Table II). In both the women with previous gestational diabetes and the control subjects one of nine insulin values increased significantly after hormonal intake for 2 months (tEe 45-minute and 150-minute values, respectively). The 120-minute plasma insulin value at the 6-month test was significantly higher than the pretreatment value in the women with previous gestational diabetes. However, no differences in the areas under the curves were observed in either group during the study (Table II).

Before treatment plasma glucagon values and the areas under the curves were identical in the warden with previous gestational diabetes and the control subjects (Fig. 1 and Table I). During treatment to changes in plasma glucagon were observed, although the suppression of glucagon was slightly more discernible in the women with previous gestational diabetes than in the control subjects as judged by the changes in the areas under the curves (Table II).

Plasma levels of free fatty acids, triglycerides, total cholesterol, and high-density lipoprotein, low-density lipoprotein, and very-low-density lipoprotein caclesterol. Before and during treatment no differences in plasma free fatty acids were observed between in women with previous gestational diabetes and the coatrol subjects (Table III). During treatment, an incresse in plasma triglycerides was found in the control subjects. Before treatment, total plasma cholescrol and high-density lipoprotein, low-density lipoprotein, and very-low-density lipoprotein cholesterol corcertrations were similar in both groups, and the corcertrations remained unchanged during treatment. Tais was also reflected in a constant high-density lipoprate n cholesterol/total cholesterol ratio in both groups during the study (Table III).

Comment

In earlier studies on oral contraceptives containing 50 µg of estrogen and having a high progestogen montent an overall risk of 44% for developing an oral gu-

cose tolerance test showing diabetes has been registered in women with previous gestational diabetes.¹⁴⁻¹⁶ Prescription of the traditional oral contraceptive compounds has therefore to a large extent been avoided in these women.

In the present study no changes in oral glucose tolerance test results were found in either women with previous gestational diabetes or in the control subjects during a 6-month period when a low-dose triphasic compound was taken. None of the women with previous gestational diabetes developed a worsening of the oral glucose tolerance test results during the treatment despite higher than normal glucose levels before entering the study. The significance of the slight increase in a few of the plasma insulin concentrations observed during the oral glucose tolerance tests in both groups is opposed by the unchanged areas under the curves. The differences observed in the decremental glucagon areas under the curves between the women with previous gestational diabetes and the control subjects may possibly reflect differences in pancreatic α-cell response to oral glucose, but the interpretation is blurred because of the marked scattering of the individual plasma values.

We have previously reported a study where a lowdose monophasic combination of 30 µg of ethinyl estradiol and 150 µg of levonorgestrel was administered to women with previous gestational diabetes. We found a more marked increase in the insulin response to oral glucose in these women than in the women with previous gestational diabetes in the present study. 17 As the progestogen component in combination compounds seems to be mainly responsible for the influence on glucose homeostasis,18 this increase might have been due to the higher progestogen dosage in the monophasic compound. Only a few other studies have dealt with the influence on glucose metabolism of low-dose oral contraceptives in women with a previous abnormality of glucose tolerance. Spellacy et al.19 studied 24 women who took a monophasic contraceptive contain-

^{†0} versus 2 months: p < 0.05.

 $[\]pm 0$ versus 6 months: p < 0.05.

ing 30 µg of ethinyl estradiol and 400 µg of norethindrone for 6 months. Two of 19 women with a normal control oral glucose tolerance test exhibited a slight deterioration in test results; five women with previous borderline abnormal control glucose tests all demonstrated improvement during 6 months of treatment. Pyörälä et al.20 investigated the effect of treatment with progestogen only (50 µg of norethindrone and 30 µg of lynestrenol) in women with previous gestational diabetes. Intake of norethindrone-containing compounds was followed by a slight deterioration of glucose tolerance whereas no changes were observed following ingestion of the lynestrenol compounds.

The mechanism behind the influence on glucose metabolism of contraceptive steroid compounds is not yet known in detail, but there is evidence that progestogens alone or combined with estrogens induce a decrease in tissue sensitivity to insulin. 18, 21, 22 Moreover, the increased plasma cortisol levels found in women treated with oral contraceptives may impair glucose tolerance as the result of an increase in hepatic glucose production and an inhibition of glucose uptake in peripheral cells.23

Since Wynn et al.24 demonstrated elevated triglyceride levels during oral contraceptive treatment, various changes in the levels of lipids and lipoproteins have been reported.4 Besides an increased plasma triglyceride concentration, oral contraceptive use is also associated with an increase in low-density lipoprotein cholesterol, very-low-density lipoprotein cholesterol, and total cholesterol, whereas the high-density lipoprotein cholesterol concentration might be decreased. These changes are positively associated with the quantity of the estrogen component although the progestogens may also modify these values depending on their biochemical constitution.5.6 Raised total cholesterol, triglyceride, low-density lipoprotein cholesterol, and very-low-density lipoprotein cholesterol levels are positively associated with the risk of developing atherosclerotic circulatory diseases, whereas an inverse relationship with the high-density lipoprotein cholesterol concentrations exists.25

The reports on the influence of the triphasic ethinyl estradiol/levonorgestrel compound on lipid/lipoprotein levels in nondiabetic women have been promising as the high-density lipoprotein cholesterol/total cholesterol ratio is unaffected by the treatment,6 but the increase in plasma triglyceride found in the present study among the control subjects is consistent with results that indicate that triphasic compounds raise plasma triglycerides to a greater extent than the low-dose monophasic ethinyl estradiol/levonorgestrel combinations.6 It is remarkable, however, that in our study the triglycerides remained unchanged in the women with previous gestational diabetes during the period of hormonal treatment and that free fatty acids also remained stable. The plasma lipids were thus unaffected by the hormonal intake, despite the elevated glucose levels. Furthermore the unchanged high-density lipoprotein, lowdensity lipoprotein, and very-low-density lipoprotein cholesterol levels together with a constant high-density lipoprotein cholesterol/total cholesterol ratio in both groups appear favorable from a clinical point of view.25

The laboratory findings in the present study are thus consistent with population studies indicating a link betweer, the epidemiologic characteristics of the oral contraceptives and their dosage and biochemistry.26 Although long-term metabolic side effects should also be excluded, the relatively progestogen-deficient triphasic preparations seem suitable as safe contraception even in women with a previous deterioration of glucose tolerance during pregnancy.

The skilled technical assistance of Lene Poulsen, Connie Kühl, Marie-Louise Borgen, and Birgitte Kiær: kov Foss is gratefully acknowledged. The contraceptive compound Triquilar was kindly supplied by Schering, Copenhagen, Denmark.

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α-Thalassemia hydrops feta_is: Clinical and ultrasonographic considerations

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Five pregnant Southeast Asian women presenting during a 14-month period with microcytic anemia, preeclampsia, and size-date discrepancies were all ultimær diagnosed as carrying fetuses with homozygous α-thalassemia hydrops fetalis. The perinatal complications of this hemoglobin disorder are unique to persons of this ethnic background and include to born fatality for the affected infant, maternal preeclamptic morbidity, and retained placenta. In this reportere obstetric ultrasound findings are presented and the clinical manifestations are discussed, with recommendations made to reduce this emerging public health problem in the United States. (AM J OBSTET GYNEC=L 985;153:500-4.)

Key words: α-Thalassemia, hydrops fetalis, Bart's nemoglobin disease, obstetric ultrasound, preeclampsia

The genetically determined disorder of hemoglob n production α-thalassemia, in the homozygous state of Bart's hemoglobin hydrops fetalis, results in uniform fatality for the affected fetus. Let In addition, the metaers of these fetuses are at high risk for severe morbidity.

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The problems resulting from this disorder are relatively unique to Southeast Asians.^{2, 3} In the United States, screening of Southeast Asians has revealed incidence rates of up to 11% for α -thalassemia trait and up to 39% for inherited hemoglobin disorders in general.^{3, 4}

Hydrops fetalis from immunologic and nonimmunologic causes often represents end-stage disease in the fetus. 5 Nonimmunologic causes of hydrops fetalis have become more conspicuous in the face of good screening and prophylaxis for rhesus-immunization disease. Homozygous α -thalassemia in the fetus presents as a non-

Table I. Maternal clinical characteristics

Patient No.	Gestational weeks on admission (menstrual age)	Fundal height (cm)	Mean arterial blood pressure (mm Hg)	Proteinuria	Deep tendon hyperreflexia	Edema	Spontaneous labor and delivery	Retained placenta	Placental weight (gm)
7.1	33	33	107	1+	2+	1+	Yes	Yes	1200
9	35*	29	103	1+	3+	1+	Pitocin augmentation	Yes	1130
3	27	33	111	3+	4+	4+	Yes	No	830
4	29	35	123	2+	4+	4+	No	Cesarean section	1320
5	29	32	97	1+	3+	3+	Yes	Yes	1030

^{*}Fetal death documented 2 weeks earlier.

Table II. Hematologic parameters*

		Maternal							
Patient No.	Hematocrit (%)	Mean corpuscular volume (fl)	Mean corpuscular hemoglobin (pg)	Paternal (fl)					
1	27.9	66	23	64					
2	45.8	64	21.5	64					
3	30.9	65	22.7	65					
4	28.0	72	23.3	64					
5	31.8	68	21.9	64†					

^{*}All maternal hemoglobin electrophoreses were normal. †Hemoglobin H disease.

immunologic cause of hydrops, and when evaluated by sonography, it is indistinguishable from other causes of hydrops fetalis. Previously reported series of sonographically determined nonimmune hydrops fetalis showed α -thalassemia to be the cause in 0% to 10% of cases, depending on the population studied.⁶⁻⁹ Many of the detected cases were in known carriers of the α -thalassemia trait prior to ultrasound examination.

We report here on five pregnant Southeast Asian women who presented to our hospital during a 14-month period with preterm labor, size-date discrepancies, preeclampsia, and hydrops fetalis. All were ultimately diagnosed as carrying fetuses with homozygous α-thalassemia. Recommendations for obstetric management of this disease are made.

Patients and methods

Records of the five patients who presented to the New York Infirmary–Beekman Downtown Hospital labor and delivery suite with nonimmune hydrops fetalis during a 14-month period were reviewed retrospectively for the first three cases and prospectively for the last two cases. These women were all from mainland China and constituted 0.7% of the total 767 patients of Southeast Asian origin who were delivered of their infants at this institution between January 1, 1984, and February 28, 1985. Two of the five patients received prenatal care from neighborhood private physicians, and two from an affiliated Chinatown community

Table III. Ultrasound findings

		Patient No.	
	3	4	5
Presentati o n	Breech	Breech	Vertex
Amniotic fluid	Absent	Severely diminished	Increased
Fetal ascites	4.	+	+ 1
Pleural effusion	+	+	→ 3,7
Skin thickness (cm)	0.8	0.7	0.7
Placental thickness (cm)	9.2	14.3	7.6
Fetal movements	None	Few	Many
Biparietal diameter (cm)	5.6*	5.3*	†
Head perimeter	22.9	22.8	+
Abdominal perimeter	31.8	28.3	

^{*}Marked dolichocephaly.

clinic. One patient, a recent immigrant, had not registered for prenatal care.

Real-time ultrasound examinations were performed in the labor room with ADR 2130 and/or General Electric Dataline equipment. Evaluations of neonatal blood for Bart's hemoglobin were provided by the Centers for Disease Control, Atlanta, Georgia. Restriction endonuclease mapping of the α - and ζ -globin genes was performed on all parents and some affected neonates. Deoxyribonucleic acid was prepared from buffy coats of peripheral blood collected in citrate. Blot hybridization with α - or ζ -globin—specific probes was carried out on deoxyribonucleic acid cleaved with Bam HI, as previously reported. ¹⁰

Results

The clinical characteristics of the gravid women on admission are summarized in Table I. Table II shows the hematologic parameters of the women and their husbands. Table III displays the obstetric ultrasonographic findings for the last three patients, those in whom adequate studies were performed.

Restriction endonuclease mapping of the α -thalassemia determinants proved the parents to be α -thalassemia carriers because of the deletion of two of four globin genes. The exception was the husband of Patient

[†]Unable to obtain correct diameters but head perimeter was noted to be much smaller than abdominal perimeter.

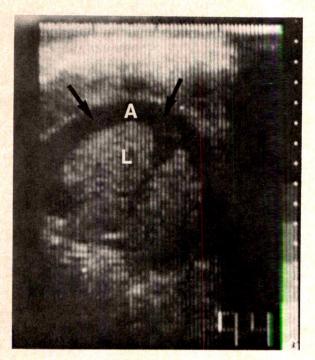


Fig. 1. Real-time sonogram showing cross section of hydropic fetal abdomen. *Arrows* point to ascitic fluid (A) outlining enlarged fetal liver (L).

5, who was found to have three of four gene deletions, resulting in hemoglobin H disease. The five infants were diagnosed as having homozygous α-thalassemia hydrops fetalis, that is, four of four gene deletions, on the basis of gene mapping studies and/or hemoglobin electrophoresis showing Bart's hemoglobin.

Brief reports of the clinical courses of the five gravid women, in order of their presentation, follow.

Case reports

Case 1. A 24-year-old woman, gravida 2, para L was admitted in labor at 33 weeks' gestation, after an unremarkable prenatal course. One year earlier she had been delivered of a healthy male infant without complications. On admission, the blood pressure was 140/90 mm Hg and she had peripheral edema (1+) and proteinuria (1+). Examination showed a gravid uterus consistent with dates and a fetal heart rate of 140 bpm. The cervix was fully dilated with the fetal vertex at the +2 station. The patient was rapidly delivered of a hydropic female infant weighing 1800 gm, with Apgar scores of 1 and 1 at 1 and 5 minutes, who died 20 minutes after birth. The third stage of labor was complicated by a retained placenta, necessitating manual removal and uterine curettage.

The mild preeclampsia resolved spontaneously, and the patient was discharged 48 hours after delivery.

Case 2. A 30-year-old woman, gravida 5, para 2, presented in early labor at 35 weeks' gestation. She had first been seen at 8 weeks by her private physician. An obstetric ultrasound at 17 weeks was performed because of a uterine size slightly smaller than dates. The

biparietal diameter of the single fetus was normal for the gestational dates, and no abnormalities were noted.

The prenatal course continued unremarkably until 2 weeks prior to admission, when she noted no fetal movements for 2 days. A real-time ultrasound showed no fetal cardiac motion, and a diagnosis of fetal death was made. A complete obstetric ultrasound examination was not performed at that time. The decision was made to await the onset of spontaneous labor, which occurred 16 days later.

Her past obstetric history revealed a stillbirth at 7 months, 8 years earlier, in Vietnam. Her second pregnancy, also in Vietnam, 7 years earlier, ended in a forceps delivery of a reportedly "brain-damaged" infant. No other details of these events were available. Two subsequent pregnancies were spontaneously aborted in the first trimester.

Physical examination on admission showed a blood pressure of 130/90 mm Hg, a gravid uterus smaller than dates, and a cervix 2 cm dilated and 100% effaced, with intact membranes and vertex presentation. There was deep tendon hyperreflexia (3+), edema (1+), and proteinuria (1+).

After amniotomy and Pitocin augmentation, the patient was delivered vaginally of a stillborn female infant, weighing 1290 gm. The placenta was retained and was removed manually. The patient was discharged 3 days later in satisfactory condition.

Case 3. A 20-year-old woman, gravida 1, para 0, presented in premature labor at 27 weeks' gestation. She had been in the United States for 1 month and had received no prenatal care. She reported an uncomplicated pregnancy until 8 days previously, when she began having swollen ankles, diarrhea, headache, and backache.

Blood pressure was 150/100 mm Hg on admission, with pitting peripheral edema (3 + to 4+), proteinuria (3+), and hyperreflexia (3+to 4+). The fundal height was 33 cm, with palpable contractions every 4 minutes. The fetal heart rate was 140 bpm. The cervix was 3 cm dilated and 90% effaced, with intact membranes and the fetus presenting by the breech at the -3 station.

Laboratory studies included a platelet count of $234,000/\text{mm}^3$ and normal coagulation and electrolyte profiles. The results of a real-time sonogram appear in Table III. Fig. 1 is a cross section of the fetal abdomen, showing the marked ascites outlining the fetal liver. All findings were compatible with α -thalassemia hydrops fetalis.

The patient was given intravenous magnesium sulfate and transferred to a level 3 perinatal facility because of prematurity. After being advised of the poor prognosis for the fetus, she decided against operative delivery. A 1490 gm stillborn infant with ambiguous genitalia was delivered vaginally. Autopsy revealed a 46,XY karyotype. The patient's severe preeclampsia improved post partum and she was discharged 4 days later in stable condition.

Case 4. A 26-year-old woman, gravida 1, para 0, was referred at 29 weeks' gestation with complaints of swollen extremities, dizziness, and upper abdominal

pain for 2 days. She had experienced a 20-pound weight gain during the preceding weeks. Otherwise, the pregnancy was complicated only by a 2 cm pedunculated genital condyloma, which was excised at 24 weeks.

On admission, the blood pressure was 150/110 mm Hg. There was marked facial edema and peripheral edema (4+). Hyperreflexia (3+ to 4+) was evident, with one beat of clonus, and she had proteinuria (2+). The fundal height was 35 cm, with no detectable contractions. The fetal heart rate was 130 bpm. The cervix was long and closed, and a floating breech presented.

The platelet count was 209,000/mm3 and coagulation studies were normal. Subsequent TORCH (toxoplasmosis, rubella virus, cytomegalovirus, and herpesvirus) studies were negative. A real-time sonogram was obtained; the results, as shown in Table III, were again consistent with α-thalassemia hydrops fetalis.

The diagnosis of severe preeclampsia at 27 weeks was made. Intravenous magnesium sulfate, a 2 gm bolus followed by three gm/hr, was administered. The fetal heart rate remained reactive at 120 to 130 bpm. Progressive oliguria ensued, without response to fluid bolus. The central venous pressure was 10 to 12 mm H₂O. For maternal indications, delivery was accomplished by a low-flap transverse cesarean section with the patient under epidural anesthesia. A grossly hydropic infant weighing 1361 gm, with ambiguous genitalia and Apgar scores of 2 and 1 at 1 and 5 minutes, respectively, was delivered. The infant died 10 minutes after birth. Autopsy was refused by the family.

The patient's postoperative course was complicated by blood pressure of 170 to 180/110 to 120, and Apresoline was added to the magnesium sulfate therapy for the first 24 hours post partum. She slowly improved during an 8-day period and was sent home in stable condition.

Case 5. A 27-year-old woman, gravida 1, para 0, with a gestation of 29 weeks by dates presented in labor and complaining of ankle swelling during the past several weeks. Two weeks previously, her private obstetrician noted a rise in blood pressure from a recent value of 90/50 to 120/80 mm Hg. Bed rest at home was prescribed, and in the ensuing 2 weeks the patient gained 19 pounds. At the onset of labor she came to the labor and delivery suite.

On admission, the blood pressure was 130/80 mm Hg and she had edema (3+), hyperreflexia (3+), and proteinuria (1+). The fundal height was 32 cm with uterine contractions occurring every 4 minutes. The fetal heart rate was 120 to 130 bpm. The cervix was 4 cm dilated and 100% effaced, with intact membranes and the fetal vertex presenting at the -1 station. Findings on real-time ultrasonography are displayed in Table III.

The diagnosis of probable α-thalassemia hydrops fetalis complicated by mild preeclampsia was made. Magnesium sulfate, 2 gm/hr, was administered intravenously and the patient's condition was stabilized. Labor progressed rapidly, and 31/2 hours after admission, she was delivered spontaneously of a live female infant,

with Apgar scores of 2 and 2 at 1 and 5 minutes, respectively, and weighing 1260 gm. The infant died after I hour and 52 minutes of life, despite intubation and resuscitative efforts.

The placenta was retained, requiring manual removal. The patient's preeclampsia resolved slowly post partum, and she went home on the third postpartum day in stable condition.

Comment

In this review of five cases of α -thalassemia hydrops fetalis occurring in New York City, the recurrent maternal presentation we noted was consistent with that in Southeast Asian reports on this condition1.2 and with the obstetric literature on nonimmune hydrops fetalis of all causes.8,9 Specifically, these women presented with varying degrees of preterm preeclampsia, microcytic anemia, and size-date discrepancies. That microcytosis is an indication of the α-thalassemia carrier state in Southeast Asians is well documented and preeclampsia is a recognized complication of nonimmune hydrops fetalis in general. However, a review from Thailand² notes its occurrence in only 50% of cases with fetal Bart's hemoglobin. We noted this condition in all of our patients. Also, the potential severity of the preeclampsia with its resultant maternal morbidity, as seen in two of the five patients here, has not been mentioned in the Western literature.

Another characteristic seen in these women, size-date discrepancy, has not been emphasized in the literature, probably because hydramnios is only occasionally present. However, size-date discrepancies were the initial indication for sonographic assessment in 46% of the patients in a recent investigation on nonimmune hydrops by Holzgreve et al.9 In Southeast Asian women with microcytosis and this finding, α-thalassemia hydrops fetalis should be ruled out. Sonography is an invaluable tool in our ability to make this diagnosis.

Of interest in our group of patients is the lack of a relationship between the progression or severity of the hydrops and the onset of labor. Three of the five initially presented in active labor, the length of which varied with parity. One woman, with the most severe preeclampsia, did not have labor at all. The gestational age at delivery in patients without a prenatal diagnosis of this condition, as reported in other studies,3.9 correlates well with gestational ages of our patients who were delivered from 27 weeks onward.

In three of the four patients delivered vaginally, the third stage of labor was complicated by retention of the enlarged bulky placenta. Although no patient suffered an obstetric hemorrhage, it is important in these cases to anticipate this complication by assuring the availability of cross-matched blood.

The presence of ambiguous genitalia is a finding that has not been previously reported in the literature on

infants with α -thalassemia. The relationship may be sporadic here, but because it was noted in two of our five cases, we will be interested in checking for this association in the future.

The sonographic findings in the three cases that were adequately evaluated met the criteria of Fleischer et al.⁷ for hydrops fetalis in general, namely, (1) presence of fetal serous cavity effusions (Fig. 1), (2) fetal anasarca (skin thickness >5 mm), (3) abnormally thick placenta (>6 cm), and (4) hydramnios or oligohydramnios. As anticipated, we could not detect an ultrasound characteristic that was specific for homozygous α -thalassemia as a cause of hydrops.

When the biophysical status of the affected fetus was assessed with ultrasound, those with oligohydramnios had a marked decrease in fetal movements; fetal breathing movements were not seen in any of the three fetuses.

To avoid the late diagnosis and maternal sequelae of α-thalassemia hydrops fetalis, antenatal screening for the carrier state in women of Southeast Asian origin (meant to include southern mainland China, Indochina, Indonesia, and the Phillippines3) is mandatory. Two good screening protocols have been published, by Stein et al.3 and Alger et al.11 Both rely on initially identifying the high-risk patient with microcytic anemia (mean corpuscular volume <82 fl) not due to simple iron deficiency. When this is found, the husband should be expeditiously evaluated for the same carrier status. Hemoglobin electrophoresis can help rule out 6-thalassemia, the other possible cause. Genetic counseling should be provided to explain the 25% risk of producing a homozygous fetus when both parents are carriers. The techniques of restriction endonuclease gene mapping are now available and this diagnosis can be made prenatally on fetal cells obtained via amni@centesis or chorionic villus biopsy.1, 10, 12 A first- or secondtrimester termination in cases of a fetus with Bart's hemoglobin will prevent the maternal morbidity experienced by our patients.

Recent Southeast Asian immigrants, who are settling in large numbers in urban centers such as New York City, may present several socio-cultural barriers to adequate prenatal diagnosis. Factors such as late or no registration for prenatal care, lack of understanding and acceptance of Western medical procedures, language and dialect barriers, religious sanctions against autopsy, and lack of entry into medicaid or third-pasty

payment systems interfere in varying degrees with the provision of high-technology obstetric services.

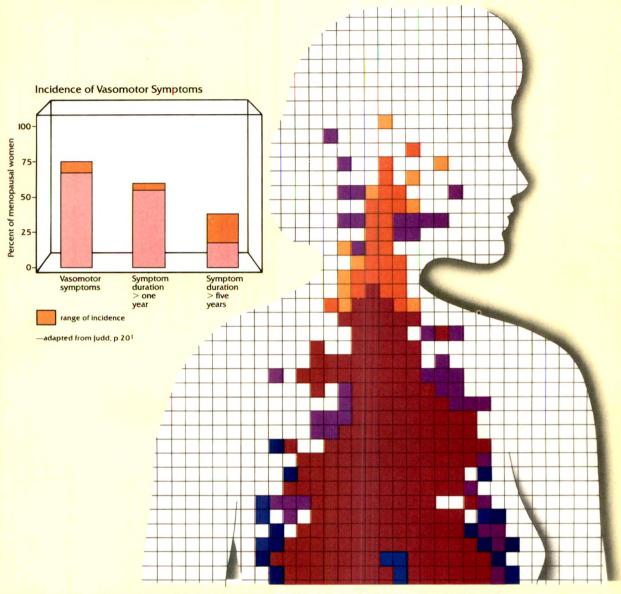
In the event that a diagnosis of α -thalassemia hydrops fetalis is not suspected until the late second or early third trimester, the following management recommendations are made. A detailed obstetric ultrasound should be performed, for detection of hydrops fetalis or other major congenital malformations. If pre-eclampsia is not severe and thus prompt delivery is not essential, amniocentesis should be performed for restriction endonuclease mapping of the α -globin genes, even late in gestation. The value in verifying this diagnosis and thus the 100% fetal mortality rate lies in our ability to then turn all concern toward the mother, to expedite delivery, and to avoid toxemic and operative morbidity.

We thank Haig Kazazian, M.D., and Corrine Boehm, M.S., of the Department of Pediatrics, Genetics Unit, The Johns Hopkins University School of Medicine, for providing the DNA restriction endonuclease studies for α -thalassemia status.

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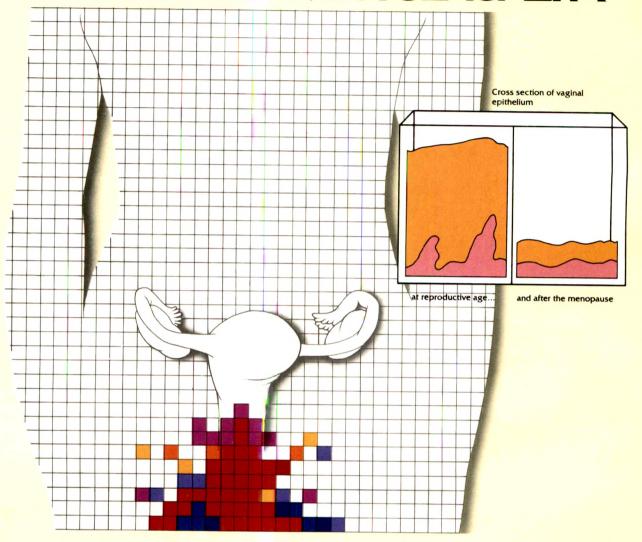




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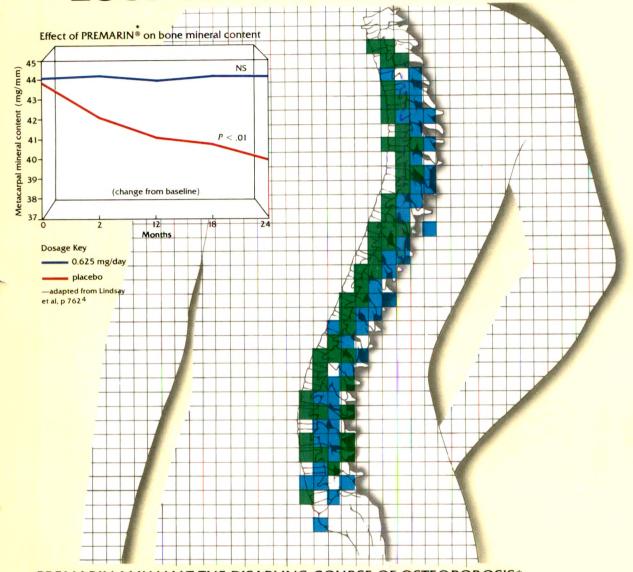
In the postmenopausal woman, decreasing levels of estrogen can have devastating effects on a woman's sexual functioning. The pH of vaginal secretions rises, promoting the growth of contaminating organisms. The vaginal epithelium dries and thins, becoming susceptible to irritation, injury, and infection. Sexual relations may be difficult or impossible.

PREMARIN (Conjugated Estrogens, U.S.P.) Vaginal Cream focuses therapy at the site of the problem. Vaginal dryness is relieved, pH reverts to its normal acidity, and the epithelium thickens and becomes more resistant to injury and infection. With the vaginal environment returned to its premenopausal state, sexual function may improve.

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BRIEF SUMMARY (FOR FULL PRESCRIBING INFORMATION AND PATIENT INFORMATION, SEE PACKAGE CIRCUI AR

PACKAGE CIRCULARY) PREMARIN® Brand of Conjugated Estrogens Tablets, U.S.P. PREMARIN® Brand of Conjugated Estrogens, U.S.P. Vaginal Cream in a nonliquefying base

ESTROGENS HAVE BEEN REPORTED TO INCREASE THE RISK OF ENDOMETRIAL ARCINOMA.

Three independent case control studies have reported an increased risk of endometrial cancer in postmenopausal women exposed to exogenous estrogens for more than one year. This risk was independent of the other known risk factors for endometrial cancer. These studies are further supported by the finding that incidence rates of endometrial cancer have encessed sharply since 1969 in eight different areas of the United States with population-based cancer reporting systems, an increase which may be related to the rapidly expanding useon "estogens during the last decade. The three case control studies reported that the risk oftendemetrial cancer in estrogen users was about 4.5 to 13.9 times greater than in nonusers. The risk appears to depend on both duration of freatment and on estrogen does. In view of these findings, when estrogens are used for the treatment of menopausal symptoms, the lowest dose that will-control symptoms should be discontinued as soon as possible. When prolonged treatment is medically indicated, the patient should be reassessed on at least a semiannual basis to determine the need for continued therapy. Although the evidence must be considered preliminary, one study suggests that cyclic administration of low doses of estrogen may carry less risk than continuous administration; it therefore appears prudent to attice such a regimen. Close clinical surveillance of all women taking estrogens is important. In allicases of undiagnosed persistent or recurring abnormal vaginal bleeding, adequate diagnostic measures should be undertaken to rule out malignancy. There is no evidence at present that "natural" estrogens are more or less hazardous than "synthetic" estrogens at equiestrogenic doses.

2. ESTEROGENS SHOULD NOT BE USED DURING PRECNAMON. Three independent case control studies have reported an increased risk of endometrial cancer

sures should be undertaken to rule out malignancy. Inere is no evidence at present trial matural" estrogens are more or less hazardous than "synthetic" estrogens at equiestrogenic doses.

2. ESTROGENS SHOULD NOT BE USED DURING PREGNANCY.
The use of female sex hormones, both estrogens and progestogens, during early pregnancy may seriously damage the offspring. It has been shown that females exposed in utero to diethylstilbestrol, a non-steroidal estrogen, have an increased risk of developing at later life a form of vaginal or cervical cancer that is ordinarily extremely rare. This risk has been estimated as not greater than 4 per 1000 exposures. Furthermore, a high percentage of such exposed women (from 30 to 90 percent) have been found to have vaginal adenosis, epitheliaschanges of the vaginal and cervix. Although these changes are histologically benign, it is not known whether they are precursors of malignancy. Although similar data are not available with the used other estrogens, it cannot be presumed they would not induce similar changes. Several reports suggest an association between intrauterine exposure to female sex hormones and congenital anomalies, including congenital heart defects and limb reduction defects. One case control study estimated a 4.7-fold increased risk of limb reduction defects in infants exposed intutero to sex hormones (oral contraceptives, hormone withdrawal tests for pregnancy, on attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb reduction defects are apposed fetuses is somewhat less than 1 per 1000. In the past, female sex hormones have been used during pregnancy in an attempted to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well controlled studies that progestogens are effective for these uses. If PREMARIN is used during pregnancy or if the patent becomes p

DESCRIPTION: PREMARIN (Conjugated Estrogens, U.S.P) contains a mixture of estrogens, obtained exclusively from natural sources, blended to represent the average composition of whater derived from pregnant mares' urine. It contains estrone, equilin, and 17α -dihydroequilin, tegether with smaller amounts of 17α -estradiol, equilenin, and 17α -dihydroequilenin as salts of their sulfate

INDICATIONS: Based on a review of PREMARIN Tablets by the National Aleademy of Sciences—National Research Council and/or other information, FDA has classified the indications for use as follows:

Effective: 1. Moderate to severe vasomotor symptoms associated with the menopause. (There is no evidence that estrogens are effective for nervous symptoms or depression without associated vasomotor symptoms, and they should not be used to treat such conditions.)

2. Atrophic varianties.

- ociated vasomotor symptoms, and they should not be used to treat such conditions.)

 Atrophic vaginitis

 Kraurosis vulvae

 Female hypogonadism

 Female castration

 Primary ovarian failure

 Breast cancer (for palliation only) in appropriately selected women and mens with
- metastatic disease.

metastatic disease.

8. Prostatic carcinoma – palliative therapy of advanced disease.

9. Postpartum breast engorgement – Although estrogens have been widely used icr the prevention of postpartum breast engorgement, controlled studies have demonstrated that the incidence of significant painful engorgement in patients not receiving such hormona" herapy is low and usually responsive to appropriate analgesic or other supportive therapy. Consequently, the benefit to be derived from estrogen therapy for this indication must be carefully engined against the potential increased risk of puerperal thromboembolism associated within heruse of large dose of estrogens.

large doses of estrogens.

Premarin has not been shown to be effective for any purpose during pregnancy and its use may cause severe harm to the fetus (see boxed).

PREGNANCY AND ITS USE MAY CAUSE SEVERE HARM TO THE FETUS (SEE, BOXED WARNING).

"Probably" effective: For estrogen deficiency-induced osteoporosis, and only when used in conjunction with other important therapeutic measures such as diet, calcium, physmtherapy, and good general health-promoting measures. Final classification of this indication requires further investigation.

INDICATIONS: PREMARIN (Conjugated Estrogens, U.S.P.) Vaginal Cream is indicated in the treatment of atrophic vaginitis and kraurosis vulvae. PREMARIN Vaginal Cream HAS NOT BEEN SHOWN TO BE EFFECTIVE FOR ANY PURPOSE DURING PREGNANCY AND ITS USE JAM CAUSE SEVERE HARM TO THE FETUS (SEE BOXED WARNING).

SHOWN TO BE EFFECTIVE FOR ANY PURPOSE DURING PREGNANCY AND ITS USE LIMP CAUSE SEVERE HARM TO THE FETUS (SEE BOXED WARNING).

CONTRAINDICATIONS: Estrogens should not be used in women (or men) with any of the iddlowing conditions: I. Known or suspected cancer of the breast except in appropriately selected patients being treated for metastatic disease. 2. Known or suspected estrogen-dependent neeplasia. 3. Known or suspected pregnancy (See Boxed Warning). 4. Undiagnosed abnormal general Billeding. 5. Active thromboembolic disorders associated with previous estrogen use (except when used in treatment of breast or prostatic malignancy).

WARNINGS: Long term continuous administration of natural and synthetic estrogens in certain animal species increases the frequency of carcinomas of the breast, cervix, vagina, and liver There are now reports that estrogens increases the risk of carcinoma of the endometrium in mimmans. (See Boxed Warning.) At the present time there is no satisfactory evidence that estrogens given to postmenopausal women increase the risk of cancer of the breast, although a recent study has reported a 2-to 3-fold increase in the risk of surgically confirmedigams. A recent study has reported a 2-to 3-fold increase in the risk of surgically confirmedigams. A diverse effects of oral contraceptives may be expected at the larger doses of estrogen used to treat prostatic or breast cancer or postpartum breast engorgement; it has been shown thattithere is an increased risk of thrombosis in men receiving estrogens for prostatic cancer and women for postpartum breast engorgement; users of oral contraceptives have an increased risk of thrombosis, and optic neuritis have been reported in a cancer and women for postpartum breast engorgement. Users of oral contraceptives have an increased risk of thrombosis in men receiving estrogens for prostatic cancer and women for postpartum breast engorgement. Users of oral contraceptives have an increased risk of thrombosis in men receiving estrogens for prostatic cancer and

pulmonary embolism and thrombophlebitis. When doses of this size are used, any of the thromboembolic and thrombotic adverse effects should be considered a clear risk.

Benigh hepatic adenomas should be considered in estrogen users having abdominal pain and tenderness, abdominal mass, or hypovolemic shock. Hepatocellular carcinoma has been reported in women taking estrogen-containing oral contraceptives. Increased blood pressure may occur with use of estrogens in the menopause and blood pressure should be monitored with estrogen use. A worsening of glucose tolerance has been observed in patients on estrogen-containing roctontraceptives. For this reason, diabetic patients should be carefully observed. Estrogens may lead to severe hypercalcemia in patients with breast cancer and bone metastases.

PRECAUTIONS: Physical examination and a complete medical and family history should be taken prior to the initiation of any estrogen therapy with special reference to blood pressure, breasts, abdomen, and pelvic organs, and should include a Paparicolau smear. As a general rule, estrogen should not be prescribed for longer than one year without another physical examination being performed. Conditions influenced by fluid retention such as asthma, epilepsy, migraine, and cardiac or renal dysfunction, require careful observation. Certain patients may develop manifestations of excessive estrogenic stimulation, such as abnormal or excessive uterine bleeding, mastodynia, etc. Prolonged administration of unopposed estrogen therapy has been reported to increase the risk of endometrial hyperplasia in some patients. Oral contraceptives appear to be associated with an increased incidence of mental depression. Patients with a history of depression should be carefully observed. Preexisting uterine leiomyomata may increase in size during estrogen use. The pathologist should be adverted in cidence of mental depression. Patients with a history of depression should be carefully observed. Preexisting uterine leiomyomata may increase in size

1. Reduced response to metryrapone test.
g. Reduced serum friglyceride and phospholipid concentration.
h. Increased serum triglyceride and phospholipid concentration.
h. Increased serum triglyceride and phospholipid concentration.
As a general principle, the administration of any drug to nursing mothers should be done only when clearly necessary since many drugs are excreted in human milk.
ADVERSE REACTIONS: The following have been reported with estrogenic therapy, including oral contraceptives. breakthrough bleeding, spotting, change in menstrual flow; dysmenorrhea; premenstrual-like syndrome; amenorrhea during and after treatment; increase in size of uterine fibromyomata; vaginal candidiasis, change in cervical erosion and in degree of cervical secretion; cystilis-like syndrome; tenderness, enlargement, secretion (of breasts); nausea, vomiting, abdominal cramps, bloating; cholestatic jaundice; chloasma or melasma which may persist when drug is discontinued; erythema multiforme; erythema nodosum; hemorrhagic eruption; loss of scalp hair; hirsulism; steepening of corneal curvature; intolerance to contact lenses; headache, migraine, dizziness, mental depression, chorea; increase or decrease in weight; reduced carbohydrate tolerance; aggravation of porphyria; edema; changes in libido.

ACUTE OVERDOSAGE: May cause nausea, and withdrawal bleeding may occur in females.

DOSAGE AND ADMINISTRATION:

PREMARIN® Brand of Conjugated Estrogens Tablets, U.S. P.

Given cyclically for short-term use only. For treatment of moderate to severe vasomotor symptoms, atrophic vaginitis, or kraurosis vulvae associated with the menopause (0.3 to 1.25 mg or more daily).

The lowest dose that will control symptoms should be chosen and medication should be discontinued as promptly as possible.

Administration should be cyclic (e.g., three weeks on and one week off).

Attempts to discontinue or taper medication should be made at three to six month intervals.

2. Given cyclically. Female hypogonadism. Female castration. Primary ovarian fai

Female hypogonadism -2.5 to 7.5 mg daily, in divided doses for 20 days, followed by a rest period refraile hypogonadism — 2.5 to 7.5 mg daily, in divided doses for 20 days, followed by a rest period of 10 days: duration. If bleeding does not occur by the end of this period, the same dosage schedule is repeated. The number of courses of estrogen therapy necessary to produce bleeding may vary depending on the responsiveness of the endometrium.

If bleeding occurs before the end of the 10 day period, begin a 20 day estrogen-progestin cyclic regimen with PREMARIN (Conjugated Estrogens Tablets, U.S.P.), 2.5to 75 mg daily in divided doses for 20 days. During the last five days of estrogen therapy, give an oral progestin. If bleeding occurs before this regimen is concluded, therapy is discontinued and may be resumed on the fifth day of bleeding.

bender this regarded as the second of the patient. For maintenance, adjust dosage to lowest level that

will provide effective control.

Osteoporosis (It orelard progression) — 1.25 mg daily, cyclically.

3. Given for a few days: Prevention of postpartum breast engorgement — 3.75 mg every four hours for five doses, or 1.25 mg every four hours for five days.

4. Given chronically. Inoperable progressing prostatic cancer — 1.25 to 2.5 mg three times daily inoperable progressing breast cancer in appropriately selected men and postmenopausal women — 10 mg three times daily for a period of at least three months.

Patients with an intact uterus should be monitored for signs of endometrial cancer and appropriate measures taken to rule out malignancy in the event of persistent or recurring abnormal vaginal historia.

bleeding PREMARIN* Brand of Conjugated Estrogens, U.S.P. Vaginal Cream Given cyclically for short-term use only. For freatment of atrophic vaginitis or kraurosis vulvae. The lowest dose that will control symptoms should be chosen and medication should be discontinued as promptly as possible. Administration should be cyclic (e.g., three weeks on and one week off). Attempts to discontinue or taper medication should be made at three to six month intervals. Usual dosage range: 2 to 4 g daily, intravaginally or topically, depending on the severity of the condition.

condition.

Treated patients with an intact uterus should be monitored closely for signs of endometrial cancer and appropriate diagnostic measures should be taken to rule out malignancy in the event of persistent or recurring abnormal vaginal bleeding.

HOW SUPPLIED: PREMARIN (Conjugated Estrogens Tablets, U.S.P.) No. 865—Each purple tablet contains 2.5 mg in bottles of 100 and 1,000. Also in Cycle Pack of 21 and in unit dose package of 100. No. 864—Each white tablet contains 0.9 mg in bottles of 100. Also in Cycle Pack of 21. No. 867—Each maroon tablet contains 0.625 mg in bottles of 100. Also in Cycle Pack of 21. No. 867—Each maroon tablet contains 0.625 mg in bottles of 100. Also in Cycle Pack of 21. No. 867—Each maroon tablet contains 0.625 mg in bottles of 100 and 1,000. Also in Cycle Pack of 21. No. 867—Each green tablet contains 0.3 mg in bottles of 100 and 1,000. The appearance of these tablets is a trademark of Ayerst Laboratories.

PREMARIN (Conjugated Estrogens, U.S.P.) Vaginal Cream—No. 872—Each gram contains 0.625 mg Conjugated Estrogens, U.S.P. (Also contains cetyl esters wax, cetyl alcohol, white was glycery monostearate, propylene glycol monostearate, methyl stearate, phenylethyl alcohol, sodium lauryl sulfate, glycerin, and mineral oil.)

Combination package: Each contains Net Wt. 1½ oz, (42.5 g) tube with one calibrated plastic applicator.

Nso Available - Refill package: Each contains Net Wt. 1½ oz. (42.5 g) tube.

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Microinvasive carcinoma of the vagina: A distinct clinical entity?

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Six patients with superficially invasive squamous carcinoma of the vagina are described. All had <2.5 mm of invasion as measured from the surface, lacked involvement of the lymph-vascular spaces, and arose within a field of carcinoma in situ. Three of the six had previously been treated for carcinoma of the cervix. The patients with microinvasive carcinoma had a median age 10 years younger than that of patients with Stage I carcinoma of the vagina. Treatment of the six patients has been by partial or total vaginectomy. With follow-up of 51 to 172 months, there have been no recurrences. More experience is needed to define microinvasive squamous carcinoma of the vagina and to determine the optimal treatment for these lesions. (Am J OBSTET GYNECOL 1985;153:505-7.)

Key words: Microinvasion, vagina, carcinoma

Carcinoma of the vagina is an infrequent disease, making up approximately 2% of admissions to a gynecologic oncology service. Although it has been well described in its intraepithelial and frankly invasive forms, no attention has been directed toward superficially invasive or "microinvasive" disease. With other squamous carcinomas of the lower genital tract, it has been possible to define an early invasive lesion that can be treated in a less radical fashion than that used for frankly invasive lesions.¹⁴

We recently reported the University of Michigan experience with carcinoma of the vagina.⁵ Among patients with Stage I disease, the 5-year survival was only 57%. Most of the patients were treated with irradiation but we noted a subgroup of patients with early disease, treated surgically, who seemed to have a better survival. This article presents six cases of superficially invasive squamous carcinoma arising within vaginal intraepithelial neoplasia, all of which were treated by partial or total vaginectomy. The pathologic features of these cases are reviewed and suggestions made for future investigation in this area.

Methods and material

The Tumor Registry at the University of Michigan Medical Center was searched for cases of carcinoma of the vagina between the years of 1950 and 1983. Hematoxylin and eosin-stained slides on all Stage I neo-

plasms were reviewed. Whenever necessary, additional slides were prepared from the blocks. All the slides were reviewed by a single gynecologic pathologist. Patients were considered for inclusion in this study if they had complete excision of the lesion by either partial or total vaginectomy. Patients having an incisional biopsy followed by radiation therapy were not included. Cases were included where there was <3 mm of invasion as measured from the overlying surface to the deepest portion of invasion. Life-table survival projections were plotted by means of the technique of Kaplan and Meier. Differences in survival were tested for significance with the generalized Wilcoxon test.

Results

During this 30-year period, 86 cases of invasive carcinoma of the vagina were treated at the University of Michigan Medical Center. There were 32 cases of Stage I carcinoma, including seven adenocarcinomas and 25 squamous carcinomas. Among the 25 squamous carcinomas, six patients met the definitions required for inclusion in this study (Table I).

The median age of these patients was 49 years (range 43 to 76 years). Five of the six were white, and one was black. Three of the six patients had a prior intraepithelial or invasive carcinoma of the cervix, and two had undergone prior pelvic irradiation. There was a predominance of involvement of the vaginal apex, although there were lesions in other portions of the vagina. Depth of invasion ranged up to 2.5 mm. None of the patients showed involvement of the lymph-vascular spaces. Patient No. 4 had a confluent carcinoma. All six lesions arose within an area of surrounding intraepithelial neoplasia (Fig. 1).

Four of the patients were treated by a total vaginectomy, one was treated by partial vaginectomy, and one

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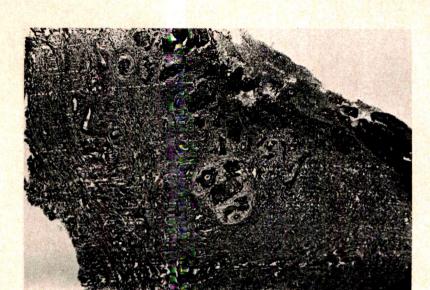


Fig. 1. Tongues of invasive squamous carcinoma reaching a depth of 1.0 mm from the surface. Note the surrounding in situ carcinoma.

Table I. Seven cases of "microinvasive" carcinoma of the vagina at the University of Michigan Medical Center

Patient	Age (yr)	Other malignancies (time interval)	Prior operation or irradiation	Location	Depth of invasion (mm)	Therapy	Follow-up	
1 60 Lymp		Lymphoma (4 yr)	Vaginal hysterectomy	Upper third anterior wall	0.8	Partial vaginectomy	133 mo, NED	
2	46	Invasive carcinoma of cervix (7 yr)	Pelvic irradiation	Apex	0.9	Total vaginectomy	133 mo, NED	
3	44	CIN (5 yr)	Abdominal hysterectomy	Apex	1.0	Total vaginectomy	62 mo, NED	
4	43	None	Abdominal hysterectomy	Apex	2.4	Total vaginectomy Vaginal irradiation and with ovoid	172 mo, NED	
5	76	None	Abdominal hysterectomy	Lower posterior	1.0	Total vaginectomy	51 mo, NED	
6	52	Invasive carcinoma of cervix (6 yr)	Pelvic irradiation	2 lesions: Upper third anterior wall and middle third posterior wall	2.5	Total vaginectomy	132 mo, NED	

CIN = Cervical intraepithelial neoplasia; NED = no evidence of disease.

was treated by a partial vaginectomy followed by superficial irradiation from a vaginal source. No attempt was made to treat the pelvic nodes, either surgically or with irradiation. Follow-up ranges from 51 to 172 months, and no patient has had a recurrence.

In comparison with the patients with Stage I squamous carcinoma of the vagina from the University of Michigan Medical Center, the patients with microinvasion had a median age that was 14 years younger. They had a similar racial distribution and a similar incidence of prior cervical carcinoma and irradiation. The survival of the patients with "microinvasive" carcinoma of the vagina and all other Stage I patients is depicted in Fig. 2. There is a statistically significant difference in survival probability (p < 0.05).

Comment

The most common model for the development of an invasive squamous carcinoma pictures the malignancy as arising first as an intraepithelial process. After either a transformation in the tumor's basic character and/or a change in the host's resistance or other modifying factors, the tumor begins to grow in an invasive pattern with the potential to invade adjacent organs and metastasize by the lymphatic and venous systems. Most cervical squamous carcinomas fit this model, but a significant proportion of invasive vulvar malignancies are felt to begin de novo as invasive lesions rather than to arise in an intraepithelial process. In the vagina the relative frequency with which intraepithelial neoplasia becomes invasive or the relative proportion of invasive

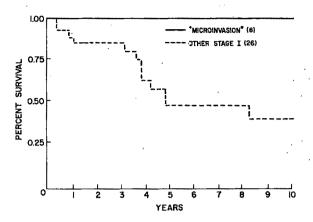


Fig. 2. Life-table survival probability for the six cases of microinvasive carcinoma compared with that of 26 cases of other Stage I carcinoma of the vagina.

lesions that arise from an intraepithelial process is not known.

With both cervical and vulvar squamous carcinoma, a great deal of attention has been directed toward identifying early invasive lesions that can be treated with less radical procedures. At the University of Michigan Medical Center, we have viewed squamous carcinomas of the cervix with <3 mm of invasion from the overlying epithelial surface and lacking confluence or lymphatic or vascular involvement as "microinvasive" and feel that these may be treated by a total hysterectomy.1 Vulvar lesions that do not show lymph-vascular involvement and with <2 mm of invasion (as measured from the surface), regardless of confluence, and lesions with: up to 3 mm of invasion that lack lymph-vascular involvement and confluence are viewed as microinvasive and are treated by wide local excision.2 Others have stated that surrounding vulvar intraepithelial neoplasia is necessary to make the diagnosis of microinvasive disease.3

Standards do not exist for the diagnosis of a microinvasive lesion in the vagina, and in presenting these six cases, we must fall back on the experience with cervical and vulvar disease. Unique problems exist in the vagina, most notably the very small deep stromal margins that can be obtained anteriorly and posteriorly, even with radical vaginectomy. It should be pointed out, however, that these anterior and posterior margins are

the same regardless of whether the procedure is considered to be a "total" vaginectomy or a "radical" vaginectomy. The total depth of vagina excised in vaginectomy specimens that we have examined varies from 0.6 to 1.2 cm. If one accepts a definition of up to 2.5 mm of invasion for microinvasive disease, then the tumor-free margin will vary from 3.5 to 12 mm. All of our cases showed surrounding intraepithelial neoplasia, and based on others' experience with vulvar carcinoma, this may be a significant predictor of a low incidence of recurrence or metastatic disease.3 The lymphatic metastatic potential of vaginal carcinoma in general is not well established, but based on experience gained from cervical and vulvar carcinoma, nodal metastasis would seem unlikely in lesions with <3 mm of invasion arising in a field of in situ carcinoma and lacking confluence or lymph-vascular space involvement.

It is not our intent to define diagnostic criteria or specific therapy for microinvasive vaginal carcinoma. Rather, we wish to suggest that there is a distinct group of patients with early vaginal squamous carcinoma who could be managed with conservative treatment. There is a void in our knowledge of early vaginal carcinoma and we wish to encourage others to critically review and report their experiences.

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The influence of antecedent renal disease on pregnancy

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The influence of antecedent renal disease on pregnancy vas studied retrospectively in 72 women with various renal diseases that had been proved by biopsy £mong 105 pregnancies studied, normal deliveries were observed in 74 (71%), abnormal deliveries with line in and in 14 (13%), fetal or neonatal deaths in 11 (10%), and spontaneous abortions in six (6%). The neidence of normal delivery, as well as that of live births, was the highest in the cases of membranous gl+ regulonephritis, but there was no obvious difference in the incidence among IgA nephropathy an ron-IgA proliferative glomerulonephritis. Cases in which there were tubulointerstitial changes of the cortical area or arteriolosclerosis in biopsy specimens and cases that included hypertension (>140/90 mm Ha or decreased renal function (glomerular filtration rate, <70 ml/min) were clearly associated with an unfe-crable outcome in delivery. It was concluded that assessment of the advisability of pregnancy in nephritica-omen should be made on the basis of a combination of the clinical and histologic parameters. (= J OBSTET GYNECOL 1985;153:508-14.)

Key words: Renal disease, pregnancy

The reciprocal influence of pregnancy and ar exedent renal parenchymal disease represents one If the greatest concerns among clinicians, especially nephrologists and obstetricians. Some of the literature has confirmed that maternal or fetal mortality and mo-dity are increased when the serum creatinine concentation is in excess of 1.6 mg/dl' or when pregnant = omen have so-called active renal diseases.24 The opining has been expressed that pregnancy may aggravate the underlying renal diseases and that the gestation ray be complicated even when the renal lesions ar∈ minimal.5,6 However, others have reported that premancy might be well tolerated in women when they have preserved renal function with normal blood pressure and when proteinuria or abnormalities of the uring y sediment are the only manifestations of their re-al involvement.7-11 The outlook of pregnancy in nephritic patients still remains a matter of debate. From this viewpoint we carried out a retrospective study in observe the relationship between antecedent renal parenchymal diseases, proved by biopsy, and the remits of pregnancies.

Patients and methods

The present study included 72 women with anderlying primary glomerular diseases during their preg-

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nancies. These patients were referred to Keio University Hospital or one of its affiliated hospitals, Toho University Hospital, Tokyo Jikeikai Medical University Hospital, National Ohji Hospital, or Osaka University Hospital, for diagnostic evaluation of chance proteinuria and/or hematuria, chronic glomerulonephritis, or nephrotic syndrome as well as for administration of adequate treatment or follow-up. In 46 cases, precise renal checkups including percutaneous renal biopsy had been performed before pregnancy and the subjects became pregnant during the follow-up period. In the remaining 26 cases, renal biopsy was carried out after delivery or abortion, since the patients had a previous history of renal disease including persistent proteinuria and/or hematuria subsequent to pregnancy.

The clinical data pertaining to the past history, serial physical examinations, and laboratory data-including renal function tests as well as clinical courses before, during, and after pregnancies-were collected, where available, from the referring doctors of each hospital and analyzed. Information was thus obtained from clinical charts of a total of 105 pregnancies. Cases in which the patients received elective abortions were excluded in this study. Cases with a diagnosis of preeclampsia without underlying renal disease, based on clinical and renal histologic findings, were also excluded.

In all cases the renal tissues were examined by light microscopy. In two thirds of the cases, electron microscopic observations and immunofluorescent studies were also carried out. The renal histologic diagnosis and precise evaluations were done by one of us (H.S.), a renal pathologist, in accordance with the criteria of the World Health Organization's histologic classifica-

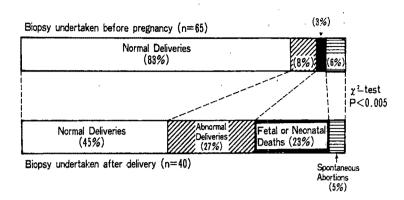


Fig. 1. Relationship between time of renal biopsy and outcome of pregnancy.

Table I. Relationship between antecedent renal disease and outcome of pregnancy*

			Deliveries			Fetal or neonatal deaths		Spontaneous abortions		
	No. of pregnant women	No. of pregnancies	Normal		Abr.ormal†					
Histologic diagnosis			n	%	n	%	n	%	n	%
IgA nephropathy	30	38	28	74	5	13	4	10	1	3
Proliferative glomeru- lonephritis (non-IgA)	23	35	25	71	6	17	2	6	2	6
Minimal change glo- merulonephritis	10	17	10	59	2	12	2	12	3	17
Membranous glomeru- lonephritis	7	13	11	84	1	8	1	8		
Membranoproliferative glomerulonephritis	1	. 1					I	100		
Sclerosing glomerulo- nephritis	I	1					1	100		
Total	72	105	74	71	14	13	11	10	6	6

^{*}Values for individual patients are available upon request.

tion of renal diseases.12 All histologic materials were obtained from the referring hospitals.

For statistical analysis, t tests and χ^2 tests were used.

Results

The 105 pregnancies were observed in 72 gravida patients with a variety of underlying renal parenchymal diseases. Table I indicates the relationship between the antecedent renal disease and outcome of pregnancy.

Histologically, one third of the patients had IgA nephropathy, and proliferative glomerulonephritis other than IgA nephropathy was the second most common diagnosis. Minimal-change glomerulonephritis and membranous glomerulonephritis followed successively. A small number of cases with membranoproliferative glomerulonephritis and sclerosing glomerulonephritis was included in this study.

The outcome of delivery was classified into four categories, that is, normal delivery, abnormal delivery with a live infant, fetal or neonatal death, and spontaneous abortion, as indicated in Table I. Abnormal delivery included cases with low-birth weight infants, premature delivery, polyhydramnios, premature abruptio placentae, and severe bleeding after delivery and are listed at the bottom of Table I.

The incidence of normal delivery in membranous glomerulonephritis was the highest, reaching 84%. In IgA nephropathy and proliferative glomerulonephritis, the ratio was between 71% and 74%. Minimalchange glomerulonephritis followed successively.

The overall incidence of abnormal delivery with live infants was 13%, but the ratio in membranous glomerulonephritis was the lowest. In membranoproliferative glomerulonephritis and sclerosing glomerulonephritis, no live birth was recorded.

The overall ratio of fetal or neonatal deaths was 10% and of spontaneous abortions 6%, respectively, in the present study. Except for the cases with membrano-

[†]Low-birth weight infants, premature delivery, polyhydramnios, premature abruptio placentae, and severe bleeding after delivery in live infants.

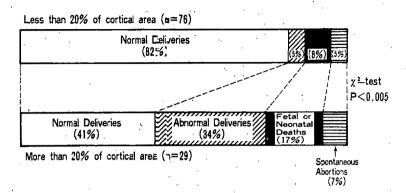


Fig. 2. Relationship between percentage of the cortical area with tubulointerstitial changes and outcome of pregnancy.

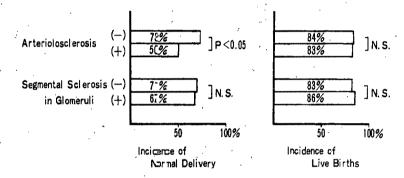


Fig. 3. Relationship between histologic factors and outcome of delivery.

proliferative glomerulonephritis and sclerosing glomerulonephritis, the percentage of fetal or neonatal deaths, as well as spontaneous abortions, was somewhat higher in minimal change glomerulonephritis, although the causes could not be clarified.

The results of the pregnancies were clearly different according to the time of renal biopsy, as shown in Fig. 1. Cases in which renal biopsy was performed before pregnancy, with careful follow-up, revealed clearly lower incidences of unfavorable outcomes as compared to cases in which biopsy was carried out after delivery or abortion in order to assess the nature of the persistent proteinuria or underlying renal diseases, irrespective of the histologic diagnosis. The data presented indicate the importance for female nephritic or proteinuric patients of childbearing age of having checkups before pregnancy and of being observed and checked regularly during pregnancy.

As to the histologic findings, important information was gained not only from the glomeruli but also from the tubules, interstitium, and arterioles. Fig. 2 shows the relationship between the percentage of the cortical area with tubulointerstitial changes and the results of pregnancy. Changes such as tubular atrophy, interstitial cell infiltration, and fibrosis in the renal cortex would

reveal the nephron injuries. Cases with tubulointerstitial changes involving more than 20% of the cortical area had obviously higher incidences of abnormal deliveries and fetal or neonatal deaths compared to cases with less tubulointerstitial changes. This difference was statistically significant.

Obvious arteriolosclerosis in the interlobular arteries or afferent arterioles and segmental sclerosis in the glomeruli were observed in nine and 15 cases, respectively. Absence of arteriolosclerosis was associated with a higher incidence of normal delivery, as demonstrated in Fig. 3, but in this study segmental sclerosis had no prognostic value for the outcome of pregnancy.

Clinically normotensive women, as well as those having preserved renal function, experienced a favorable outcome in delivery. As illustrated in Fig. 4, patients with normal blood pressure (<140/90 mm Hg) had statistically higher rates of normal delivery (77%) and of live births (88%) when compared with those of hypertensive cases (50% and 71%, respectively). With regard to renal function, the rate of normal delivery was 76% in the cases with good renal function (that is, glomerular filtration rate of >70 ml/min or serum creatinine level of <1.1 mg/dl), whereas the incidence decreased to 36% in the cases with impaired renal func-

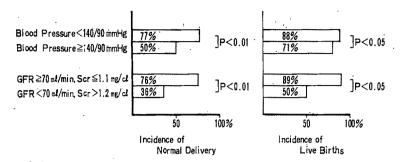


Fig. 4. Relationship between clinical factors and outcome of delivery.

Table II. Summary of histologic as well as clinical factors in cases in which pregnancy ended in fetal and neonatal deaths

Patient No.	Histologic diagnosis	Tubulointerstitial changes (>20%)	Hypertension (>140/90 mm Hg)	Decreased glomerular filtration rate (<70° ml/min)
A-11	IgA nephropathy, mild	******	· +	+
B-2	IgA nephropathy, mild		+	_
B-4	IgA nephropathy, moderate	+ .	· _	_
A-12	IgA nephropathy, advanced	+	+ .	+
A-35	Proliferative glomerulonephritis, mild		+	-
B-11	Proliferative glomerulonephritis, mild with focal glomerular sclerosis	+	. +	+
B-18	Minimal change glomerulone- phritis	+	+	_
B-20	Minimal change glomerulone- phritis	*****		+
B-22	Membranous glomerulonephritis	****	+	-
B-25	Membranoproliferative glomer- ulonephritis		+	_
B-26	Sclerosing glomerulonephritis	+		+

Cases in which + was indicated in hypertension or decreased glomerular filtration rate include those that had high blood pressure or renal function impairment during the observation period before delivery.

tion. Therefore these criteria might mark the limits for occurrence of a normal delivery.

Table II summarizes certain clinical and histologic factors in 11 cases whose pregnancies ended in fetal or neonatal deaths. Except for a few cases, the glomerular changes were not necessarily severe, but all of these cases showed at least one of the following: tubulointerstitial changes involving >20% of the cortical area, a blood pressure of >140/90 mm Hg and a glomerular filtration rate of <70 ml/min.

Table III presents the cases in which renal function decreased during pregnancy or after delivery. According to this table, with the exception of three cases, pregnancy might have an unfavorable irreversible effect on the clinical course of the underlying renal diseases. Most of these cases showed good renal function before pregnancy but had either marked tubulointerstitial changes or high blood pressure or both. The above-

*

mentioned data clearly indicate the importance of such factors; that is to say, a combination of histologic as well as clinical factors should be taken into consideration in assessing the advisability of pregnancy in nephritic patients.

Comment

It has ong been debated whether women with underlying renal parenchymal disease may tolerate pregnancy well or not. Recently the dominant opinion has been that such women may have a favorable result of pregnancy when they have good renal function with normal blood pressure and when proteinuria or abnormalities of the urinary sediment are the sole manifestations of their renal diseases. This may be true in many cases, but at the same time, as Fairley et al. and Kincaid-Smith have pointed out, we sometimes experience a complicated outcome of pregnancy even

Table III. Summary of histologic and clinical actors in cases in which renal function decreased during pregnancy or after delivery

Patient No.	Histological diagnosis	Tubulo- interstitial changes (>20%)	Art=rivlo- scl=rc=is	Hyper- tension (>140/90 mm Hg)	Renal function			
					Before pregnancy	During pregnancy	After delivery	Outcome of pregnancy
A-3	IgA nephropathy, mild	+			Normal	Normal	Ţ	Spontaneous abortion
A-14	IgA nephropathy, mild	+	+	. —	Normal	Normal	1	Acute renal failure
A-21	IgA nephropathy, mild	+		· +	Normal	Ţ	ļ	Premature delivery
B-4	IgA nephropathy, moderate	-+-		· —	Normal	ţ	Normal	Acute renal failure fetal death
A-12	IgA nephropathy, ad- vanced	+	+	+	Normal	Ţ	\downarrow	Fetal death
B-15	Proliferative glomeru- lonephritis, mild	+	* ,	. +	Normal	1	Normal	Acute renal failure
B-11	Proliferative glomeru- lonephritis, mild with focal glomeru-	+		+	Normal	1	1	Spontaneous abortion
	lar sclerosis	•		•				
A-36	Proliferative glomeru- lonephritis, moder- ate	-	+ **:	+ ,	1	Ì	1	Normal delivery
A-46	Membranous glomeru- lonephritis			+	Normal	\downarrow	Normal	Premature twins
B-25	Membranoproliferative glomerulonephritis	_	***************************************	+	Normal		\downarrow	Fetal death
B-26	Sclerosing glomerulo- nephritis	+	· +		1	\downarrow	1	Fetal death

^{*}Arrow indicates decreased renal function.

when there is good renal function with minimal histologic findings. Such cases often plague ciricians when they are assessing the advisability of pregnancy in nephritic patients. The present study was undertaken to gain more information concerning the relationship between antecedent renal diseases proved by biopsy and the outcome of pregnancies, as well as the effect of pregnancy on maternal renal diseases.

As indicated in Table I, the overall incidence of normal delivery and that of live births were 71% and 84%, respectively. These incidences were nearly the same as those reported by Katz et al.9 In this study, th∈ cases in which the patient received an elective abortion were excluded, since it was difficult to clarify the real circumstances. According to the clinical charts of these cases, however, 19% of the total 129 pregnarcies underwent this procedure. If these cases were included, the rate of normal delivery and that of live birtLs would be reduced to 55% and 68%, respectively. Someo them were evaluated as having a higher possibility o zrious risk by continuing the pregnancies, but others, probably one half, underwent the procedure only because of anxiety. Therefore adequate renal checkurs before conception are recommended in order to a od undergoing this unnecessary procedure.

From the statistics issued by the Japanese Ministry of Health and Welfare, 13 the incidence of fetal and neo-

natal deaths between 1972 and 1982 amounted to about 4%, including spontaneous abortions. This clearly indicates a higher risk during pregnancy in nephritic women as a whole. However, the risk may be diminished to approximately 9% by performing checkups before pregnancy and assessing the advisability of pregnancy, as shown by the data in Fig. 1.

Histologically, the incidence of normal delivery, as well as that of live births, was highest in membranous glomerulonephritis, but there were no obvious differences in incidence among IgA nephropathy and non-IgA proliferative glomerulonephritis when the patients were in a stable condition. Minimal change glomerulonephritis followed successively. No live births were recorded among the patients with membranoproliferative glomerulonephritis and sclerosing glomerulonephritis, although the number of these patients was small. Few studies have been published concerning pregnancy in women with membranoproliferative glomerulonephritis, 5. 9, 14 and further studies are needed to assess the advisability of pregnancy under these conditions. We do not, however, recommend that such patients carry through with the pregnancy, because of the generally poor prognosis of membranoproliferative glomerulonephritis reported in the literature.14, 15

There were several cases showing marked tubulointerstitial changes in the renal cortex or arteriolosclerosis, in spite of mild glomerular abnormalities. Tubulointerstitial changes in the cortical area would reveal the degree of nephron injuries. It is reported that the degree of such changes might correlate with an increase in the serum creatinine level or a decrease in the glomerular filtration rate in primary glomerular diseases.16 When a sample of renal tissue was obtained by biopsy needle, and when the number of glomeruli that could be observed was limited, the degree of tubulointerstitial changes was a very important factor in estimating the nephron injury if interstitial nephritis was ruled out.

Histologic damages sometimes precede the decline of renal function or rise of blood pressure, which makes such findings valuable in providing us with important information concerning the outlook for the clinical coures, especially when there are discrepancies between the clinical data and histologic findings.

Clinically, blood pressure and renal function are very important factors in assessing the possibility of normal delivery, as shown in Fig. 4. Although there were a few exceptional cases, a blood pressure of 140/90 mm Hg and glomerular filtration rate of 70 ml/min would mark the limits for expecting a favorable outcome of delivery. A serum creatinine level of 1.1 mg/dl is equivalent to a glomerular filtration rate of 70 ml/min in the average Japanese woman of childbearing age. Serum creatinine levels may differ according to the muscular mass of the patients, but they do represent one of the important laboratory indices. From our experience the upper limit of 1.5 mg/dl reported by Bear¹ appears to be a little too high to permit normal delivery, since this value would be equivalent to a glomerular filtration rate of 50 ml/min.

It is a very important problem for clinicians to decide into which levels the nephritic patients should be divided when assessing normal and complicated pregnancies. According to previous medical papers, a serum creatinine level of 1.1 mg/dl or glomerular filtration rate of 70 ml/min seems too rigid, but we would like to insist on these levels in order to reduce the possibility of an unfavorable outcome in delivery or of further negative influence on the underlying renal diseases.

In this study, renal function decreased during pregnancy in 11 cases. Among them, three patients recovered to the level of that before pregnancy, but eight had persistent renal impairment after delivery or abortions (Table III). From these data it is difficult to conclude whether the intervening pregnancy might have negative influence on the antecedent glomerular diseases or whether they showed only the natural progression of the original diseases regardless of the preg-

In evaluating the patient's prognosis as well as the

advisability of pregnancy, we would like to stress again that the clinician ought to make assessments from a combination of the clinical and histologic findings. On the basis of the above discussion, it may be concluded that the criteria necessary to achieve a normal birth in nephritic women are as follows: (1) Clinically the blood pressure must be <140/90 mm Hg, the glomerular filtration rate >70 ml/min, and serum creatinine level <1.1 mg.dl before pregnancy. (2) Histologically the disease must be membranous glomerulonephritis, IgA nephropathy (mild), diffuse proliferative glomerulonephritis (mild), or minimal change glomerulonephritis in remission, with tubulointerstitial changes involving less than 20% of the cortical area and without obvious arterioloxclerosis. Conditions that might be associated with serious results are nephritides with moderate or severe glomerular injuries, or mild glomerular abnormalities with tubulointerstitial changes involving more than 20% of the cortical area and/or with obvious arteriolosclerosis, along with high blood pressure (>140/90 mm Hg) and decreased renal function (<70 ml/min).

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Plasma prorenin in first-trimester pregnancy: Relationship to changes in human chorionic gonadotropin

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Prorenin and human chorionic gonadotropin are both anthesized in chorionic cells. The relationship of changes in maternal plasma prorenin to changes in himman chorionic gonadotropin were therefore evaluated during the first trimester. In samples submitted to the routine chemistry laboratory for detection of pregnancy a positive relationship was observed between prorenin and β human chorionic gonadotropin during the 5 weeks following conception. Subsequently human chorionic gonadotropin continued to rise but prorenin had reached a plateau. Serial studies in one subject demonstrated that prorenin had increased to 65% of maximum by the thirteenth day following conception whereas human chorionic gonadotropin had risen to only 0.2% of maximum. By 3 to 5 days post to tum, β human chorionic gonadotropin had fallen by 98% but prorenin had fallen by only 50%. The early rise in prorenin following conception and the relatively slow fall post partum suggest that pregnancy-related analyses in maternal plasma prorenin are of maternal, not fetal, forigin. (AM J OBSTET GYNECOL 1985;153:514-3)

Key words: Prorenin, inactive renin in pregnancy human chorionic gonadotropin

Prorenin is an inactive form of the enzyme regin that normally circulates in human blood in concentrations close to 10 times that of active renin. All of the active renin in blood from nonpregnant subjects appears to be derived from the kidney since it is virtually absent in anephric subjects. In contrast, although protein is probably, in part, of renal origin, it is always present in the blood of anephric subjects, usually at subspirmal levels, and is therefore secreted into the circulation from both renal and extrarenal sources.

Prorenin is synthesized in the chorion^{3, 4} by the cytotrophoblastic cells,⁵ the same cells that synthe i.e human chorionic gonadotropin,⁶ and it has been reported that prorenin is released in vitro from both the maternal and the fetal sides of these cells.⁷ Abnormal—high

levels of prorenin occur in maternal plasma during pregnancy, 8-11 about tenfold higher than normal, and even higher levels are found in amniotic fluid. 12 Nonetheless, it is not known whether the increase in maternal prorenin is due to increased secretion from the kidney, from the fetoplacental unit, or from another source.

The purpose of the present study was to evaluate the time course of changes in plasma prorenin early in pregnancy and to determine whether there is a relationship between the circulating levels of human chorionic gonadotropin (hCG) and prorenin in the maternal circulation. In fact they were positively related but only during the first 5 weeks. The prorenin concentration in maternal plasma reached a peak several weeks before that of hCG, suggesting that it may be one of the earliest hormones to reach maximal levels during pregnancy. The pattern of the fall in prorenin post partum suggests that its source is maternal rather than fetal.

Material and methods

Patients. Thirty-four serum samples that had been collected for measurement of the β -subunit of hCG (β -hCG) were obtained from the routine chemistry labo-

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Table I. Laboratory values in two patients evaluated sequentially

Gestation*	Plasma renin activity† (ng/ml/hr)	Prorenin‡ (ng/ml/hr) 、	Prorenen V _{nas} § (ng/ɔɪl/l-r)	Renin substrate (ng/ml)	β -hCG (mIU/ml)
Patient 1			-	-	
13 days	8.3	78	164	2440	118
14 days	3.3	67	150		164
16 days	6.9	105	228	2310	388
28 days	8.1	124	250	3154 ["]	13,600
5 wk	6.5	133	220	4110	43,200
6 wk	4.7	130	222	3820	57,200
7 wk	4.1	135	222	4200	78,400
8 wk	4.8	132	226	3780	61,000
Postpartum	0.7	16.3	58	2051	<u>.</u>
Patient 2	•				
Preconception	1.4	6.8	21	1271	_
4 wk	5.3	68	183	1389	8,690
5 wk	2.9	68	182	1722	12,640
6 wk	6.1	85	193	2206	30,990
7 wk	4.8	95	211	2207	_
8 wk	4.0	99	201	2624	63,660
9 wk	4.5	92	192	2481	43,660
10 wk	8.9	92	.196	2375	<u>.</u>
ll wk	6.0	102	18-2	3425	32,240
12 wk	4.1	119	200	3976	33,960
13 wk	8.3	121	254	2880	29,070
14 wk	6.6	114	187	4203	18,740

^{*}Gestation was calculated from estimated date of conception.

|Renin substrate was estimated from the mean of days 13 and 14.

ratory. The charts of all the patients were reviewed to determine the date of the last menstrual period and only those samples from patients with no known abnormality were assayed for prorenin. Gestational age was calculated from 14 days after the beginning of the last menstrual cycle. Therefore 2 weeks' gestation, for example, means 4 weeks since the last menstrual period or approximately 2 weeks following conception.

Renin system and β-hCG measurements were also carried out in sequentially collected samples during the first trimester of pregnancy from two normal subjects starting 13 days and 4 weeks, respectively, after conception.

To evaluate the time course of the fall in β-hCG and components of the renin system, measurements were also performed on samples collected from five additional patients during the thirty-fifth to thirty-ninth week of gestation, 3 to 5 days post partum, and again 5 to 6 weeks post partum. One patient had transient pregnancy-induced hypertension, one had chronic hypertension, one had mild preeclampsia, and two had chronic hypertension with superimposed preeclampsia. The patient with transient hypertension had a cesarean section. The rest had normal spontaneous vaginal deliveries.

Processing of blood. Blood from the routine laboratory was centrifuged at room temperature but the sera were often subsequently stored in a refrigerator for a few days before they were stored frozen at -40° C. Blood from the other patients was collected into ethylenediaminetetraacetic acid (EDTA) Vacutainers and centrifuged at room temperature and the plasma immediately frozen.

Hormonal measurements. We have previously shown that incubation of plasma in the refrigerator does not result in destruction of renin or renin substrate¹³ and that renin can be assayed in either se rum or EDTA/plasma as long as 3 mmol/L EDTA is added to the serum.14

Plasma renin activity was measured by incubating the plasma or serum for 3 hours at 37° C and pH 5.7 in the presence of 3 mmol/L EDTA and 3 mmol/L phenylmethy.suifonyl fluoride (PMSF). The angiotensin I generated during the 37° C incubation was then measured by radioimmunoassay.14 Plasma renin activity is expressed as nanograms of angiotensin I generated per milliliter of plasma per hour of incubation. Prorenin was activated by incubating duplicate samples at pH 7.8 with 2.5 mg/ml of trypsin at -4° C for pregnancy samples (or 15 mg/ml for prepartum or postpartum samples) for 1 hour in the presence of 5 mmol/L benzamidine. Subsequently, 2.5 mg/ml (or 1.5 mg/ml) of soya bean trypsin inhibitor was added and the samples incubated for 15 minutes at 25° C. Then renin activity

[†]Rate of angiotensin I formed per milliliter plasma per hour of incubation with use of endogenous renin substrate.

[‡]Prorenin = total renin following trypsin activation minus plasma renin activity.

[§]Prorenin V_{max} = calculated prorenin concentration at substrate excess with use of Michaelis-Menten formula and Michaelis constant of 2700 ng/ml.

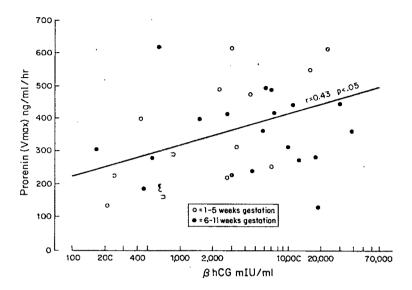


Fig. 1. Relationship between plasma β - γ CG and plasma prorenin (\overline{V}_{max}) during the first trimester of pregnancy. The correlation was significant for the group as a whole (r = 0.43, p < 0.05) and for the samples collected during the first ξ weeks (r = 0.59, p < 0.05) but not for samples collected between 6 and 11 weeks (r = 0.30).

was measured by incubating the samples at 37° C and pH 5.7 for 0 and 1 hour as described above. The "blank" in the zero time sample was subtracted from the incubated sample. The results for total renin vere then expressed as nanograms per milliliter per hour. Prorenin equals total renin minus active renin The period of refrigeration that occurred for samples from the routine laboratory most likely caused some activation of inactive renin by cryoactivation13 since the plasma renin activity of these samples was often unusually high. Normally plasma renin activity is <10 ng/ml/hr during the first trimester (see Table I and reference 16) but was above this value in 15 of the 34 samples and averaged 12.0 ng/ml/hr. If prorenin is partially activated before active renin is measured, the prorenin value will be underestimated. For this reason, we have reported herein only the total renin results for the samples from the routine laboratory. Close to 90% of the total plasma renin is in the form of prorenin in normal pregnancy.10

Renin substrate was measured by incubating plasma for 1 hour at 37° C and pH 5.7 in the presence of EDTA, PMSF, and excess human renal renin. The concentration of renin added ensured complete exhaustion of renin substrate during the 1-hour incubation. The results were expressed as nanograms angiotensin Ligenerated per milliliter plasma.

In the measurement of plasma renin activity described above the concentrations of both the enzyme renin and renin substrate are normally rate-limiting. Renin substrate concentration in normal human plasma averages 1800 ng/ml, while the Michaelis constant for human renin in human plasma is close to 2700 ng/ml.¹⁸

Therefore, changes in renin substrate concentration can markedly affect the prorenin measurement. Renin substrate increases gradually throughout gestation and is more than double the baseline level by the end of the first trimester. Therefore, to estimate changes in prorenin independent of changes in substrate concentration, we calculated the prorenin concentration at excess substrate concentration [prorenin (V_{max})] from the plasma prorenin and renin substrate levels with the Michaelis-Merten equation and a Michaelis constant of 2700 ng/ml. ¹⁸

 β -hCG was measured by a double-antibody radio-immunoassay method with the β III hCG-Beta radio-immunoassay kit from Serono Diagnostics.

Results

We confirmed in the samples from the routine chemistry laboratory that there is a temporal relationship between gestational age and the maternal plasma β -hCG during the first trimester ($r=0.56,\ p<.001$). Nonetheless, there was a wide scatter in the data that may have been related to the imprecise definition of gestational age and also to interindividual variability in the rate of increase in β -hCG. The two patients who were evaluated sequentially showed a very close relationship between β -hCG and gestational age, with the peak being reached around 7 to 8 weeks (Table I).

We found no statistically significant relationship between gestational age and prorenin (V_{max}) during the first trimester or during the first 5 weeks following conception. Also the relationship between gestational age and β -hCG was not significant for the first 5 weeks. Despite this, there was a significant relationship be-

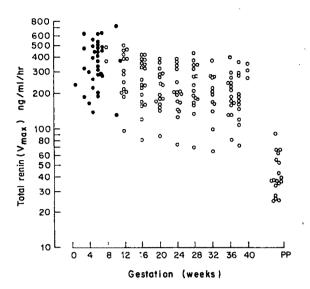


Fig. 2. Relationship between total plasma renin (V_{max}) and gestational age during the first trimester (solid symbols) and during the second and third trimesters and 6 weeks post partum (PP). The solid symbols represent data collected in this study, and the open symbols are derived from data previously reported in reference 10. Prorenin could not be reliably measured in the samples represented by solid symbols because they were stored in a refrigerator, which causes cryoactivation of prorenin. Therefore, total renin data are presented. Prorenin accounts for approximately 90% of the total renin in pregnant subjects.

tween log β -hCG and prorenin (V_{max}) during the first trimester (Fig. 1; r = 0.43, p < .05). Further analysis showed that the relationship was significant only for samples collected during the first 5 weeks (n = 13, r = 0.59, p < 0.05) but not for samples collected between 6 and 11 weeks (n = 21, r = 0.30, NS). This early relationship most likely occurred because maternal plasma prorenin was increasing during the first 5 weeks but had reached its peak by 5 weeks' gestation.

The total renin values measured during the first trimester that were obtained in the present study were compared with the total renin measurements during the second and third trimesters obtained in a previous study (Fig. 2). The total renin (V_{max}) from the two studies was quite similar in magnitude and it is quite clear that it had reached close to its maximum value by the fourth week following conception. We clearly failed to detect the initial rise in prorenin since very few samples were collected prior to that time.

In one subject who was evaluated serially from the second week following conception we were able to observe the tail end of the rise in prorenin (Patient I, Table I). The prorenin (V_{max}) was 38 ng/ml/hr 6 weeks post partum and we assume that this was close to the baseline level prior to conception. On the thirteenth and fourteenth days following conception prorenin (V_{max}) levels were 164 and 150 ng/ml/hr, respectively.

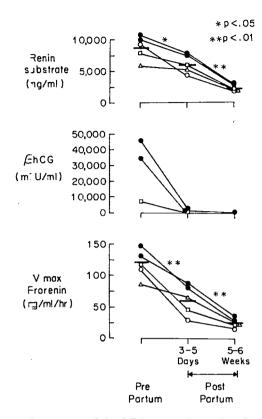


Fig. 3. Time course of the fall in prorenin, renin substrate, and β -hC \supset post partum. \circ = Pregnancy-induced hypertension; \square = chronic hypertension; \triangle = preeclampsia; \bullet = preeclampsia and chronic hypertension.

By 16 days it had increased to 228 ng/ml and remained quite constant at this level thereafter despite a further twofold increase in renin substrate (Table I). Approximately £5% of the pregnancy-induced increase in prorenin (V_{max}) had occurred by 14 days following conception, that is, before the next menstrual cycle would normally have begun, whereas only 0.2% of the increase in β-hCG had occurred. A second subject was evaluated weekly from the fourth week following conception but prorenin (V_{max}) had reached its peak prior to this time and remained remarkably stable thereafter (Table I) By the fourth week β-hCG had reached only 14% of its maximal value. In both of these subjects βhCG reached its peak at 7 to 8 weeks, that is, 3 to 4 weeks later than prorenin. After this peak the β-hCG began to fall. Thus the time course of the rise in these hormones was different, the magnitude of the increase in B-hCC was greater, and the subsequent pattern of secretion following attainment of peak values was also different

To evaluate whether the fall in maternal prorenin following delivery was consistent with it's being secreted by the fetoplacental unit, we measured the time course of the fall in maternal plasma prorenin post partum in five additional subjects and compared it to the change

in renin substrate (which is secreted by the maternal liver) and in β -hCG (which is secreted from the fetoplacental unit). Plasma prorenin (V_{max}) fell slowly in all five subjects. It averaged 119 ± 10 (SEM) ng/ml/hr prior to delivery, 59 ± 10 ng/ml/hr (50%) 3 to 5 days post partum, and 24.2 ± 3.2 ng/ml/hr 5 to 6 weeks post partum (Fig. 3). Renin substrate also fell slowly in all subjects from 8700 ± 900 to 6100 ± 700 ng/m (72%) at 3 to 5 days to 2100 ± 100 ng/ml 5 to 6 weeks post partum. In contrast β -hCG fell rapidly in the three subjects in whom it was measured and was only 2% of prepartum levels by 3 to 5 days post partum despite the fact that it was unusually high in two of these subjects immediately before delivery, most likely because they had preeclampsia. 19

Comment

In the present study we have demonstrated that during pregnancy the peak of maternal plasma p-o-enin is reached by 4 weeks' gestation, much earlier than that of β-hCG, and may have reached close to meximum before the next menstrual cycle would normall-cccur. Previous studies have shown that plasma protenin is high early in pregnancy and remains at the same high levels throughout gestation,8-11 but this is the firt study to examine the levels in a large group of subject during the first trimester and to compare them to changes in hCG. In contrast to prorenin, the peak of β-hCC occurs around the seventh to tenth week, several wee is later, and the blood level falls thereafter to about 105 of the peak value by the third trimester.6 Thus, although both of these substances are synthesized by the zyrozrophoblast,5 there are clearly marked differences_n their patterns of secretion into the maternal circulator.

We measured β-hCG in the same samples that were used for the prorenin measurement. We were able to show that β-hCG increased significantly during he first trimester. This was reassuring considering that h s was not a prospective study and the samples had mt been collected and handled under ideal conditions. Despite the observation that prorenin had reached its near by 4 weeks (Fig. 2) while β-hCG had not, we bund a statistically significant relationship between β-hDG and prorenin during the first 5 weeks of gestation. This relationship most likely occurred because bch hormones were rising during the first 5 weeks while during subsequent weeks only β -hCG continued to rise. The lack of a relationship between gestational age and plasma prorenin during the first 5 weeks could have been the result of the inaccuracies involved in the estimation of gestational age.

The question arises concerning the source of the increase in maternal plasma prorenin early in pregnancy. Although Poisner et al. were able to show that when fetal membranes were perfused in an Ussing chamber,

prorenin could be detected in the perfusate on both the maternal and fetal sides of the membrane, this does not necessarily indicate that secretion of chorionic prorenin into the maternal circulation occurs in vivo. We have shown herein that the rate of decline of plasma prorenin postpartum is slow compared to that of βhCG and can be measured in terms of days (Fig. 3). The half-life of prorenin following total nephrectomy is reported to be close to 2 hours.²⁰ Thus, if the pregnancy-related rise in prorenin had been of fetal origin, plasma prorenin should have returned to baseline by 3 to 5 days post partum. This did not occur. It can be seen in Fig. 3 that the rate of decline of prorenin post partum was instead quite similar to that of renin substrate, a substance produced by the maternal liver whose circulating level is presumably stimulated by the high estrogen levels that occur during pregnancy.16, 21 If prorenin were secreted into the maternal circulation by the fetoplacental unit, then its presence so early in gestation would suggest that it is synthesized by the blastocyst prior to implantation. If so, the amount of prorenin secreted per cell would have to decrease proportionally as maturation takes place, in order to maintain the constant levels observed in the maternal circulation from the fourth week of gestation. It is more likely, however, that prorenin is not released into the maternal circulation from the chorionic cells but passes from these cells only into the fetoplacental unit. The rise in inactive renin that occurs during pregnancy may instead be from a maternal source and be induced by pregnancy-related hormonal or physiologic changes. This is consistent with reports that showed that total renin or prorenin was not higher in uterine venous blood than in peripheral blood during cesarean section at term^{22, 23} and that the renin concentration in chorion from the fetal surface of the placenta was greater than that of the maternal surface of the placenta.3

Before the presence of prorenin was suspected in human-plasma two commonly used methods for measuring plasma renin required an acidification step to pH 3.3. We now know that these methods actually measured total renin, not active renin, since acidification pH 3.3 causes activation of inactive renin. Using these methods two groups measured changes in renin throughout the menstrual cycle and demonstrated a significant rise during the luteal phase.24.25 There are no other published data on total or inactive renin during the menstrual cycle. If 90% of the renin measured in those studies was inactive, this suggests that prorenin increases during the luteal phase of the menstrual cycle. If so, it is possible that the rise in prorenin during the luteal phase is merely extended and exaggerated following conception, which would be consistent with a maternal, rather than fetal, source of plasma prorenin during pregnancy.

Altogether, then, this study has shown that a close to tenfold rise in plasma prorenin occurs during the first 4 weeks following conception and that the increase is sustained at close to maximum values throughout pregnancy. We have also shown that the fall in prorenin post partum is much slower than has been observed following nephrectomy, suggesting that the source of the prorenin may not be the fetoplacental unit. Further studies are required to elucidate the role of prorenin during pregnancy both in the maternal circulation and in the fetoplacental unit.

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For further information see letter by Sealey et cl. cn pages 596-7.

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Histologic control of biochemical steroid receptor analysis in endometrial carcinomas

John T. Soper, M.D., Kenneth S. McCarty, Jr., M.D., Ph.D., William T. Creasman, M.D., and Kenneth S. McCarty, Sr., Ph.D.

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Histologic review of 422 specimens from endometrial carp noma submitted for biochemical cytosol estrogen and progesterone receptor analysis revealed that 16 (4.0%) contained no evidence of carcinoma on permanent histologic sections. An additional 11 (2.5%) contained focal carcinomas on permanent sections but had no evidence of malignancy in frozen technics of the tissue submitted for receptor analyses. Despite the paucity or even absence of malignancy, many of these specimens had significant cytoplasmic estrogen and progesterone receptors derived from endometrial and myometrial tissues. Rigorous histologic control of specimens from endometrial parcinomas submitted for biochemical steroid receptor analyses is necessary to establish valid clinics and histologic associations of steroid receptor content. (AM J OBSTET GYNECOL 1985;153:520-3.)

Key words: Estrogen receptor, progesterone receptor, endometrial carcinoma, steroid receptor, content of endometrial carcinoma

Since intracellular protein receptors were cemonstrated to be necessary for steroid hormonal action in steroid target tissues, receptor binding content as measured by biochemical assays has been extensively andied in malignancies of these tissues to establish almical and histopathologic correlates. The prognostic and therapeutic value for determining cytoplasmic extragen and progesterone receptor binding in breast carrinomas has been well established.1 Currently, man- intestigators are reporting histologic, clinical, and therapeutic significance for estrogen receptor and procesterone receptor binding in endometrial carcinc mas. The purpose of this communication is to draw attention to pitfalls in biochemical receptor analyses of endometrial carcinomas and the need for rigorous hi tclegic control of tissue used for these assays.

Material and methods

Tissue specimens were reviewed from all patients with endometrial carcinomas submitted to the steroid receptor laboratory for cytosol estrogen receptor and progesterone receptor analysis from its inception in 1976, through June, 1982. All patients had a previous

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Table I. Source of tissue for steroid receptor analysis in 422 endometrial carcinomas

		No carcinon in specimen		
•	Total	n	%	
Hysterectomy	368	21	6.0	
Curettage or endo- metrial biopsy	13	1	7.7	
Biopsy of metastasis	41	3	7.4	

diagnosis of endometrial carcinoma by biopsy or curettage or by frozen section during the procedure that resulted in tissue submitted for steroid receptor analyses. Specimens submitted for receptor analyses were obtained by endometrial biopsy or curettage, hysterectomy, or directed biopsy of metastatic lesions.

All permanent histologic sections of tissue submitted for receptor analyses were reviewed to confirm the diagnosis of carcinoma. Recuts of the original paraffin blocks and special stains were used when indicated. A portion of the tissue from each specimen submitted for biochemical steroid receptor analyses was frozen in liquid nitrogen and stored at -70° C (Revco freezer) for possible additional histologic studies. Sections of the frozen tissue were made with the cryostat and stained with hematoxylin and eosin if: (1) replacement of tissue by carcinoma was not confirmed, (2) only focal lesions were present, or (3) the lesion had questionable histologic features in permanent sections adjacent to material submitted for receptor analysis.

Cytosol estrogen receptor and progesterone receptor

Table II. Steroid receptor content and receptor specimen histologic results of lesions with no evidence of carcinoma on permanent section

	D	Post to the install		gen receptor mg protein)		erone receptor /mg protein)
Procedure	Permanent section histologic results	Receptor specimen histologic results	8S SDGA	DCCA	6S SDGA	DCCA
Hysterectomy	Proliferative endo- metrium	Grade 2 adenocarci- noma	23.6	ND	ND	ND
Endometrial biopsy	Endocervical glands	Grade 1 adenocarci- noma	30.2	ND	ND	ND
Hysterectomy	Adenomatous hyperplasia	Adenomatous hy- perplasia, myo- metrium	151.8	ND	14.1	ND
Hysterectomy	Adenomatous hyperplasia	Myometrium	88.0	ND	ND	ND
Hysterectomy	Adenomatous hyperplasia, deci- dualized stroma	Decidualized stroma	31.8	ND	ND	ND
Hysterectomy	Proliferative endo- metrium	Proliferative endo- metrium	45.9	868.0 $K_d 3.1 \times 10^{-10}$	ND	394.4 $K_d 1.0 \times 10^{-9}$
Hysterectomy	Proliferative endo- metrium	Proliferative endo- metrium	93.1	ND	ND	685.3 $K_d 9.4 \times 10^{-10}$
Hysterectomy	Proliferative endo- metrium	Myometrium	59.6	117.7 $K_d 1.9 \times 10^{-10}$	190.3	ND
Hysterectomy	Secretory endome- trium	Secretory endome- trium	24.3	ND	66.6	$46.4 \atop { m K_d} 2.4 \times 10^{-10}$
Vaginal biopsy	Necroțic tissue	Necrotic tissue	3.2	ND	ŊD	ND
Hysterectomy	Adenomatous hyperplasia	Insufficient tissue	136.6	184.9 $K_d 4.1 \times 10^{-10}$	235.9	1732.9 $K_d 9.4 \times 10^{-10}$
Hysterectomy	Adenomatous hyperplasia	Insufficient tissue	18.6	ND	4.7	ND
Hysterectomy	Adenomatous hyperplasia	Insufficient tissue	35.3	53.8 $K_d 2.0 \times 10^{-10}$	143.4	ND
Hysterectomy	Adenomatous hyperplasia	Insufficient tissue	71.0	ND	ND	ND
Vaginal biopsy	Inflammation	Insufficient tissue	ND	ND	ND	<1
Liver biopsy	Normal liver	Insufficient tissue	ND	<1	ND	ND

SDGA = Sucrose density gradient; DCCA = dextran-coated charcoal analysis; ND = not performed; K_d = dissociation constant.

analyses were performed as previously described.2.3 Both multiconcentration titration analysis with the use of dextran-coated charcoal and sucrose density gradient analysis were performed. Saturable binding of >10 fmol/mg of protein calculated4.5 to have highaffinity binding (dissociation constant $< 1.0 \times 10^{-9}$) by dextran-coated charcoal analysis and >7 fmol/mg of protein by sucrose density gradient analysis in the 8S region for estrogen receptor and ôS region for progesterone receptor was considered evidence of significant cytoplasmic receptor binding.

Results

Tissue specimens from 381 primary and 41 metastatic endometrial carcinomas were submitted for estrogen receptor and progesterone receptor analyses. Specimens were obtained by hysterectomy in 87%, curettage or endometrial biopsy in 3%, and directed biopsy of metastasis in 10% (Table I). In all cases, the

diagnosis of endometrial carcinoma had been confirmed histologically either at the time of operation or by previous biopsy. Endometrial carcinoma was not present in cryostat sections of the tissue submitted for receptor analysis in 21 of 368 (6.0%) specimens obtained by hysterectomy, one of 13 (7.7%) obtained by curettage, and three of 41 (7.4%) specimens obtained by biopsy of metastatic lesions.

Sixteen specimens (4.0%) submitted for receptor analysis contained no evidence of malignancy on permanent sections (Table II). Two of these specimens contained malignancy only in frozen sections of the portion submitted for receptor analysis but not in the permanent sections. Eight had no evidence of malignancy in frozen sections and six had insufficient tissue for histologic evaluation in the portion submitted for receptor analysis (Table II). Six of these patients originally had well-differentiated carcinomas with coexisting adenomatous or cystic hyperplasia. Most of these

Table III. Steroid receptor content and histologic results of lesions with malignancy documented by permanent sections

	D	7		gen receptor /mg protein)		erone receptor /mg protein)
Procedure	Permanent section histologic results	R xepter specimen Estelagic results	8S SDGA	DCCA	6S SDGA	DCCA
Hysterectomy	Focal grade 1 adenocarcinoma	Adenometeus hyperplasia	17.0	ND	ND	ND
Hysterectomy	Focal grade 1 adenocarcinoma	Adenometcus hyperplasia	130.7	ΝÖ	107.0	ND
Hysterectomy	Focal grade 2 adenocarcinoma	Adenomatous hyperplasia	<1	ND	ND	ND
Hysterectomy	Focal grade 1 adenoacanthoma	Adenometeus hyperplasia	10.5	ND	ND	ND
Curettage	Grade 1 adenoacanthoma	Adenometeus hyperplasia	ND	$\begin{array}{c} 66.8 \\ K_{\text{d}} \; 4.1 \times 10^{-11} \end{array}$	267.5	471.8 $K_d 1.0 \times 10$
Hysterectomy	Focal grade 1 adenocarcinoma, polyp	Cystic	ND	45.8 $K_d 1.0 \times 10^{-9}$	214.1	30.9 $K_d 2.5 \times 10^{-3}$
Hysterectomy	Focal grade 2 adenocarcinoma	Prolife a ve endome- trium, endocervix	101.4	ND	154.1	ND
Hysterectomy	Focal grade 1 adenocarcinoma	Prolif∈ a ive endome- triu⊓	275.8	ND	ND	ND
Hysterectomy	The state of the s	Prolife a ive endome-	73.5	ND	ND	ND
Hysterectomy	Focal grade 1 adenocarcinoma	Myom trium	ND	<1	<1	ND
Hysterectomy	Focal grade 1 adenocarcinoma	Myom trium	220.9	ND	<1	ND

SDGA = Sucrose density gradient; DCCA = dext-ar-coated charcoal analysis; ND = not performed; K₄ = dissociation constant.

specimens contained significant estrogen receptor and/or progesterone receptor: 13 of 15 (87%) had significant estrogen receptor and seven of nine (78%) had significant progesterone receptor. Among estrogen receptor—negative specimens, one contained normal hepatic tissue from a percutaneous liver biopsy and the other contained only necrotic inflammatory tis ue obtained by biopsy of a vaginal lesion. One proge terone receptor—negative specimen contained adenomatous hyperplasia and the other was composed of tissue from the vaginal nodule.

An additional 11 (3%) specimens had maligrancy documented by permanent histologic sections of tissue adjacent to the portion submitted for biochemical receptor analyses; however, these were focal carcinomas. Examination of frozen sections from the tissue submitted for receptor analyses revealed only benign adenomatous hyperplasia or normal endometria, myometrial, or endocervical tissues (Table III). Again the majority of these specimens had significant e trogen receptor and/or progesterone receptor content: nine of 11 (89%) had significant estrogen receptor and four of six (67%) had significant progesterone receptor.

Comment

The determination of cytosol estrogen receptor and progesterone receptor content by means of biochemical assays in malignancies of steroid hormone targe. t ssues

appears to give additional information regarding the functional behavior of these tumors beyond information gained by histologic or clinical evaluation.6 This has been well established for breast carcinoma.1 Studies also indicate that the estrogen receptor and progesterone receptor content of endometrial carcinomas correlates with histologic differentiation, response to hormonal therapy, and prognosis in endometrial carcinomas.2 7-13 Biochemical analyses of steroid receptor content of whole-tissue homogenates do not allow localization of receptor among tissue components. Therefore, it is important to confirm the presence of endometrial carcinoma in specimens analyzed for estrogen receptor and progesterone receptor content so that valid histologic and clinical associations can be established by biochemical assay.

A variety of techniques have been reported for ensuring histologic verification of carcinoma in endometrial specimens analyzed for receptor content.^{2, 7-12} The majority of investigators have submitted similar or adjacent samples of tissue obtained by biopsy, curettage, or hysterectomy for analysis of histologic characteristics and receptor content. Since most patients treated for endometrial carcinoma at this institution are referred for therapy following a diagnostic curettage, we have obtained the majority of tissue for receptor analysis of primary endometrial carcinomas from hysterectomy specimens. Often the gross identification of

carcinoma is difficult, especially following curettage or in focal well-differentiated carcinomas. However, as Table III illustrates, even the presence of carcinoma in sections adjacent to the tissue analyzed for receptor content does not ensure the histologic findings of the specimen actually analyzed. Since estrogen receptor and progesterone receptor are present in myometrium, normal endometrium, and hyperplastic endometrium9.11.12 the presence of appreciable estrogen receptor and progesterone receptor does not guarantee that the specimen contains carcinoma. Mortel et al.14 have recognized this as a problem of tissue heterogeneity. In contrast, breast carcinomas are surrounded by tissues containing low levels of sex steroid receptor.

Our current practice is that endometrial carcinoma specimens submitted for estrogen receptor and progesterone receptor analyses have direct histologic review of permanent sections from tissue adjacent to the specimen submitted for biochemical receptor analyses. A portion of the receptor tissue specimen is retained for frozen section analysis if the permanent sections reveal questionable or only focal malignancy. In this manner, endometrial malignancies will have an adequate, yet simple, histologic control for study of clinical and histologic correlates of estrogen receptor and progesterone receptor content when biochemical analyses are used. Definitive localization of receptor in endometrial carcinoma awaits the development and verification of immunohistologic techiques with the use of antibodies specific for purified estrogen receptor or progesterone receptor.

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Progesterone receptors in human vaginal tissue

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Specific progesterone receptors were measured in both cytoplasmic and nuclear fractions of vaginal tissues obtained from 17 women between 33 and 53 years in age. Whereas nuclear receptors could be measured in 11 of 17 tissues examined, cytoplasmic receptors were detected in only four. The distribution of receptor-positive tissues was similar in the follocular and futeal phases and no significant difference was observed in the nuclear receptor concentration in the two phases. The receptor concentration from all tissues examined ranged from 31 to 105 pmol/mg of protein and the apparent dissociation constant was 1 to 2 nmol/L. The presence of progesterone receptors in the nuclear fraction and their absence in the cytoplasm raise questions about the authenticity of the classical two-step model for steroid hormone action. (Am J OBSTET GYNECOL 1985;153:524-8.)

Key words: Progesterone receptors, vagina, nuclear receptors

It is well known that estrogen and progesterone induce specific changes in vaginal cytologic characteristics. Although the effects of progesterone are less marked or less well studied, in contrast to the effects of estrogens, change brought about by progesterone can clearly be seen during histologic examination of vaginal smears. These are marked by the presence of more intermediate cells than superficial cells, crowding of cells, and the presence of navicular cells.

The various cellular changes induced by progesterone, similar to those by estrogens, in responsive tissues are brought about by an initial interaction of the steroid molecule with its specific receptors. The mechanism of this genomic action is now fairly well understood in terms of the orthodox two-step model.1.2 According to this model the steroid, after entering the cell, first combines with its receptor in the cytoplasm, and subsequently the receptor complex moves into the nucleus where through a series of steps it leads to changes in deoxyribonucleic acid transcription. In keeping with the above mechanism of action, receptors for both estrogen and progesterone have been identified and characterized in the vaginal tissues of experimental animals. However, in a recent study on human vaginal tissue by van Haaften et al.,3 the authors reported the absence of progesterone receptors although estrogen receptors were present. These authors took great care in ruling out methodologic problems that could have been responsible for the lack of detection of progesterone receptors in the human vagina. However, they

examined only the cytosolic fractions for the presence of progesterone receptors.

In the present study, we obtained vaginal tissue from 17 women and examined both cytosolic and nuclear fractions for the presence of progesterone receptors. In the majority of the tissues nuclear but not cytoplasmic receptors were present.

Material and methods

Tissue samples were obtained from 17 women between 33 and 55 years in age. The material was collected during routine abdominal hysterectomy because of adenomyosis or myoma (10 patients) and operations for uterine prolapse (seven patients). Whereas 12 patients had regular spontaneous menstrual cycles, the remaining five were perimenopausal with menstrual irregularities or were receiving hormonal treatment.

About 1 hour before the operation, 10 ml of venous blood was drawn from each patient into heparinized tubes and centrifuged. The plasma was stored frozen at -18° C until analyzed for steroids.

Immediately after excision, the vaginal tissue and in some cases the myometrial tissue were placed in icecold 0.9% saline solution.

After removal of fat and surrounding connective tissue the pieces were washed twice with an ice-cold solution of buffer A containing 10 mmol/L Tris hydrochloride (pH 7.4) and 1 mmol/L ethylenediaminetetraacetic acid .n 1 mmol/L dithiothreitol, 10% glycerol, 0.5 mmol/L phenylmethyl sulfonyl fluoride, and 0.1 mmol/L tosylphenylalanyl-chloromethyl ketone. The last two substances mentioned were used as inhibitors of proteolysis. All subsequent procedures were performed at 4° C unless otherwise indicated.

The tissues were homogenized in about 10 volumes of the above-mentioned buffer with a Polytron homogenizer for three periods of 10 sec/gm of tissue with

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Patient No.	Age (yr)	Nuclear (fmol/mg protein)	Cytosolic (fmol/mg protein)
l	45	37.7	NS
2	45	85.6	NS
3	50	94.0	75.3
4	55	NS	NS
5	54	54.7	NS

NS = No specific binding.

intermittent pauses of 30 seconds. The homogenate was centrifuged at $1000 \times g$ for 10 minutes to obtain the nuclear pellet. The supernatant was centrifuged at $45,000 \times g$ and the pellet discarded while the cytosolic supernatant was saved. The nuclear pellet was extracted with 0.4 mol/L potassium chloride, incorporated in buffer A, for 45 minutes. The procedure for the determination of progesterone receptors in the nuclear and cytosolic fractions was essentially that described previously with some modifications.

Aliquots (300 µl) of cytosol or nuclear extract were added to assay tubes containing six different concentrations (0.3 to 2.4 nmol/L) of tritium-labeled progesterone with or without a 250-fold molar excess of nonradioactive progesterone. After 18 hours of incubation, 0.4 ml of dextran-charcoal solution (0.05% dextran 80 and 0.5% charcoal) was added to each assay tube. The contents were mixed for 5 seconds and allowed to stand for 1 minute. The tubes were then centrifuged at 1500 × g for 5 minutes. The supernatant was decanted and, after mixing with scintillation cocktail (Aquasol, New England Nuclear), 0.5 ml was counted in a Packard Tri-Carb liquid scintillation spectrometer. Protein concentration in the cytosol and nuclear extract was determined by the method of Lowry et al.⁵

Estradiol and progesterone in plasma were determined as described previously.6

Tissues from regularly menstruating women were divided into two groups with regard to the phase of the menstrual cycle, based on plasma steroid levels. The follicular phase included women with plasma progesterone levels ≤2 ng/ml and estradiol levels ≤100 pg/ml, and the luteal phase included those having progesterone levels >2 ng/ml.⁶ In all cases this grouping, according to plasma hormone levels, concurred well with the dating of the menstrual period. The mean values for the plasma estradiol and progesterone in the two groups are shown in Fig. 1.

Results

A plot of steroid binding against concentration reached saturation of specific binding sites and resulted

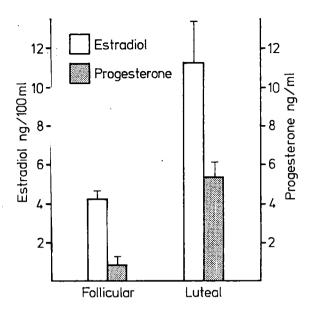


Fig. 1. Mean (\pm SEM) concentration of plasma 17 β -estradiol and progesterone in patients in the follicular (n = 5) and luteal (n = 7) phases.

in a linear Scatchard plot indicating presence of progesterore receptors (Fig. 2, A). The absence of receptors in the cytoplasmic fraction prepared from the same tissue could be confirmed by nonsaturability of the binding sites and incompatibility with Scatchard analysis (Fig. 2, B). Thus the data presented in Fig. 2 represent a typical example of vaginal tissue having nuclear receptors but lacking cytoplasmic receptors.

The data on individual analysis of tissues from patients in the follicular phase are shown in Table I and from those in the luteal phase in Table II. Patients whose data are shown in Table III either were perimenopausal or had received some form of hormonal treatment as indicated. In the follicular phase, cytoplasmic progesterone receptors could be detected in only one of five women, whereas nuclear progesterone receptors were present in four of the five with a mean density of 68 fmol/mg of protein (Table I).

In the uteal phase, two and four of seven individual tissues examined showed the presence of cytoplasmic and nuclear progesterone receptors, respectively (Table II]. The mean concentrations in the cytoplasmic and nuclear fractions were 86 and 100 fmol/mg of protein, respectively. In one patient (No. 12) from this group myometrial tissue was also analyzed for progesterone receptors. The concentrations in the cytoplasmic and nuclear fractions of the myometrium were 368 and 174 fmol/mg of protein, respectively. The corresponding values for vaginal tissue (Table II) were 105 and 103 fmol/mg of protein, respectively.

Among the five patients listed in Table III, one and three were receptor positive for cytoplasmic and nu-

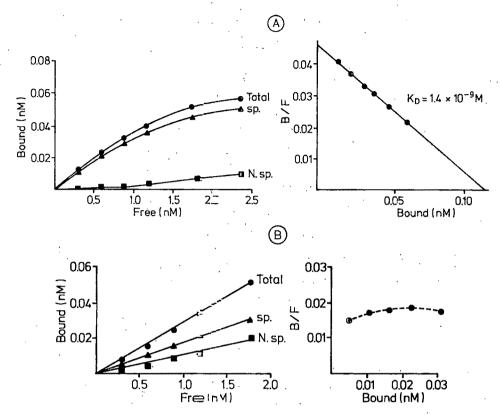


Fig. 2. Saturation and Scatchard anal six of the data on progesterone binding in nuclear (A) and cytoplasmic (B) fractions of vaginal tis us obtained from Patient No. 2.

Table II. Concentration of progesterone receptors in cytoplasmic and nuclear fractions of vaginal tissues in luteal phase

Patient No.	Age (yr)	Nuclear (fmol/mg protein)	Cytoscic (fmol/mg fro en)
6	34	59.6	96.⊐
7	42	97.8	NS ⁻
8 '	33	NS	NS
9	37	NS	· NS
10	43	86.0	NS .
11	50	NS	NS .
12	41	103.2	1053

NS = No specific binding.

clear fractions, respectively. Thus, of a total of 17 raginal tissues analyzed, only four showed the pre-exe of progesterone receptors in the cytoplasmic fraction, whereas progesterone receptors could clearly be reasured in 11 of the nuclear fractions. The appa ext dissociation constant from all receptor-positive tis use was 1 to 2 nmol/L.

Because of the relatively small number shorting the presence of receptors in the cytosolic fraction. a comparison between cytosolic and nuclear fractions could not be made. Nuclear progesterone receptor concentrations in follicular and luteal phases are compared

Table III. Concentration of progesterone receptors in cytoplasmic and nuclear fractions of vaginal tissues obtained from women with clinical status as shown under Remarks

Patient No.	Age (yr)	Nuclear (fmol/mg protein)	Cytosolic (fmol/mg protein)	Remarks
13	49	NS	NS	Perimenopausal
14	35	31.4	NS	Depo-Provera, 2 yr
15	45	NS	NS	Depo-Provera, 0.5 yr
16 .	50	60.9	72.0	Perimenopausal
17	35	68.2	NS	Depo-Provera, 1 yr

NS = No specific binding.

for four tissues, each showing the presence of receptor (Fig. 3). No significant difference was observed. There was no relationship between plasma estradiol or progesterone concentration and receptor density (data not shown).

Comment

The data of the present study provided clear evidence of the presence of progesterone receptors in human vaginal tissue. The absence of progesterone receptors in the study by van Haaften et al.³ could be explained by the fact that these authors analyzed only

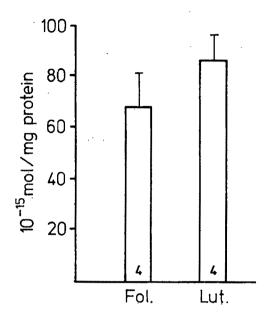


Fig. 3. Nuclear progesterone receptor concentrations in vaginal tissue in follicular (Fol.) and luteal (Lut.) phases. Values are means \pm SEM for four tissue samples in each phase.

cytosolic fractions. In the present study less than 25% of the tissues examined were found to be receptor positive for the cytosolic fractions. In comparison two thirds of the nuclear fractions analyzed exhibited the presence of progesterone receptors. Since nuclear fractions were thoroughly washed before they were assayed for receptors it is unlikely that the presence of receptors was due to contamination from the cytoplasmic proteins. On the contrary, since the number of receptor-positive samples for the cytosolic fractions was much smaller than that in the nuclear fractions, the possibility of the presence of fragments of nuclear origin in the cytosolic fraction cannot be totally excluded. Accordingly, a solid case for the presence of cytosolic receptors cannot be made.

The present finding showing the presence of progesterone receptors in the nuclear fraction, and their absence in the cytoplasmic fraction is difficult to reconcile with the generally accepted model of steroid hormone action. This is particularly difficult to reconcile with the extensive data published on the human myometrium and endometrium where no problems with the detection of progesterone receptors in cytosolic fractions seem to have been encountered.7-9 The question whether the cytoplasmic receptors result from a contamination by the nuclear fragments or represent other artifacts introduced during isolation procedures has recently been raised by some authors 10-14 who have been unable to visualize the presence of steroid hormone-binding sites with autoradiographic and immunocytochemical techniques. These and some other

studies have raised a question about the authenticity of cytoplasmic receptors and thereby cast a shadow upon the validity of the classical two-step model for sex steroid—normone action.^{15, 16}

Our results give additional support to arguments challenging the concept of the two-step model. However, the possibility that entirely different mechanisms may operate in different tissues and that the human vagina represents one example of diversion from the classical model cannot yet be ruled out. Careful studies on receptor distribution, taking into account the possibility of contamination, and rigorous analyses of receptor specificity will be needed to resolve the question of whether cytoplasmic receptors indeed are present or even, in fact, needed in mediating steroid hormone action. ^{15, 16} Human vaginal tissue in this context offers good material for further studies.

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Antepartum fetal monitoring in insulin-dependent diabetic pregnancies

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In order to minimize unexplained stillbirths in insulin-dependent diabetic pregnancies, fetal well-being was assessed by antepartum monitoring while development of pulmonary maturity was awaited. Antepartum monitoring consisted of outpatient nonstress tests beginning at 32 weeks' gestation. Fetuses with nonreactive nonstress tests were further evaluated by contraction stress tests and were delivered if tests were positive. With use of this system there were no unexpected stillbirths during management of 119 insulin-dependent diabetic pregnancies. Of 14 infants delivered because of positive contraction stress tests, six were found to have major disorders; the other explicit had no major residual neonatal morbidity. Thus this system of antepartum fetal surveillance: (1) eliminated unexplained stillbirths, (2) identified a subgroup of insulin-dependent diabetic pregnancies with a high rate of major fetal abnormalities, and (3) allowed for identification and subsequent timely delivery the other distressed fetuses that were at a high risk of neonatal morbidity and/or mortality, such that potential long-term adverse outcomes were avoided. (AM J OBSTET GYNECOL 1985;153:528-33.)

Key words: Insulin-dependent diabetic pregnancies antepartum monitoring, stillbirths, fetal anomalies, fetal distress

The optimal timing of delivery of insulin-dependent diabetic pregnancies relies upon minimization of the risks of both intrauterine fetal death and hyaline membrane disease. Historically, patients with diabetes were electively delivered at a predetermined gestational age according to White's classification of diabetes; vith more advanced diabetes, delivery was effected at at earlier gestational age. Currently, most centers try to postpone delivery until fetal lung maturity can be established, thereby nearly eliminating the occurrence of hyaline membrane disease. While the development of pulmonary maturity is awaited, antepartum surveilance is conducted in order to minimize the occurrence of unexplained stillbirths.

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Historically, antepartum testing in insulin-dependent diabetic pregnancies relied upon daily measurement of plasma and urinary estriol levels, and use of this measurement continues to be described. 2.3.5 With the advent of fetal heart rate assessment, antepartum obstetric assessment evolved into two types of testing, nonstress tests and contraction stress tests. More recently, the biophysical profile has also been introduced as a means of performing antepartum fetal monitoring, and its significance is currently being assessed. 8

This report describes a 6-year period of antepartum electronic fetal monitoring in insulin-dependent diabetic pregnancies, during which there were no unexplained stillbirths. Specifically, it describes the antepartum identification of fetal distress and the outcome of infants delivered following the detection of fetal distress.

Material and methods

The study group for this report is composed of all insulin-dependent diabetic patients who underwent antepartum electronic fetal monitoring and were subsequently delivered of their infants during a 6-year pe-

Table I. Definitions of antepartum fetal monitoring

Type of test	Criteria
Nonstress test	
Reactive	Two accelerations of fetal heart rate from baseline of 15 bpm for 15 sec each, within 10 min time period
Nonreactive	Failure to meet above criteria in 40 min
Contraction stress test	
Negative	Three contractions within a 10 min time period without associated late decelerations
Positive	Three contractions within a 10 min time period, each of which is associated with late deceleration
Equivocal	Late decelerations occurring in association with some contractions but not meeting the criteria for a positive contraction stress test

riod from January 1, 1977, to December 31, 1982, at Vanderbilt University Medical Center, a tertiary referral hospital. Data collection was accomplished by a review of the medical records of these women and their infants.

Management of insulin-dependent diabetic pregnancies has been previously described.² Briefly, the principle of obstetric care of these women has been an increasing reliance on outpatient management, while development of fetal pulmonary maturity was awaited, at which time delivery was performed. Hospital admissions were limited to those for obstetric indications or delivery.

The mainstay of the antepartum fetal monitoring system at Vanderbilt University Medical Center has been the nonstress test and the contraction stress test. The definitions used for reactive and nonreactive nonstress tests as well as negative and positive contraction stress tests are shown in Table I. Nonstress tests were initiated beginning at 32 weeks gestation and, if reactive, continued until delivery. If fetal well-being could not be established by the nonstress test (that is, a nonreactive nonstress test), a contraction stress test was performed. If the contraction stress test was negative, nonstress tests were resumed. However, a positive contraction stress test was thought to represent possible fetal distress and to be an indication for delivery.

Congenital anomalies and birth injuries were limited to those identified in the nursery. Diagnosis of neonatal respiratory distress was obtained from the neonatal intensive care unit and based on published criteria.

Pregnancies were categorized into three groups. Group I was composed of 14 pregnancies identified as

Table II. Antepartum monitoring groups

Group	Definition	No. of pregnancies
1	Positive contraction stress test—delivered	14
1 A	Positive contraction stress test—delivered, no major disorder	8
2	Negative contraction stress test	48
3	Reactive nonstress test	57
Total		119

involving fetal distress as identified by the antepartum monitoring protocol. Group 1A was composed of a subset of group 1, consisting of pregnancies delivered of infants without major disorders. Group 2 was composed of those pregnancies in which a contraction stress test was performed at some point during pregnancy but was found to be negative. Individuals in group 3 had reactive nonstress tests throughout pregnancy.

Statistical analysis was performed by the χ^2 test, Fisher's exact test, and the unpaired t test. All data were expressed as mean \pm SEM.

Results

Of the 172 insulin-dependent diabetic patients delivered at Vanderbilt University Medical Center from 1977 to 1982, 127 underwent antepartum testing with 119 assessed according to protocol. The remaining patients were managed by private practitioners and admitted for delivery or were transports who did not underge artepartum testing. The distribution of patients among the antepartum monitoring groups is shown in Table II. Among patients not managed by protocol, four hac nonreactive nonstress tests that were not followed immediately by contraction stress tests; two patients had subsequent reactive nonstress tests, and two patients had negative contraction stress tests the following cay. Also, in four patients positive contraction stress tests were not followed by immediate delivery; instead, in three women the contraction stress test was continued and became negative, while in the other patient the positive contraction stress test was performed elsewhere, with no evidence of fetal distress at our institution after transport.

There were no unexplained stillbirths in any group. The only intrauterine fetal death occurred in an infant in group 2 in a mother who developed diabetic ketoacidosis. There were two neonatal deaths in infants from groups 2 and 3, and there were severe congenital heart anomalies in each.

Table III compares the maternal demographic characteristics of the 119 pregnant patients with diabetes who underwent antepartum monitoring. The distributions of race, age, and length of pregnancy at the

Table III. Demographics in pregnant women with insulin-dependent diabetes undergoing antepartum fetal monitoring

momtoring			<u> </u>					
	Positive contracto-1 stress test			Group 2: Nonreactive nonstress test, negative contraction stress test (n = 48)		Group 3: Reactive nonstress test (n = 57)		
	Gra	$\sup 1 \ (n = 14)$	Groi	$up \ 1A \ (n = 8)$				
Characteristic	No.	Mean ± SEM	-VI-	Mean ± SEM	No.	Mean ± SEM	No.	Mean ± SEM
Age (yr)	14	27.2 ± 2.2	8	27.4 ± 2.2	· 48	26.1 ± 0.9	57	28.0 ± 0.9
Estimated gestational age at first VUH visit (wk)	14	25.2 ± 2.1	ક	23.4 ± 2.9	48	21.7 ± 1.5	56	20.7 ± 1.3
White's classification of diabetes B (gestational) B C D F G R Classes B, C Classes D, F, R	3 · 4 · 4 · 1 · 1 · 2 · 1	11 3	I W I	5 3	17 4 19 5 2 6	40 8	29 8 12 5 2 8	49 8
Race White . Black	10 · 4		≅ . ₹		40 -		48	
Complications of preg- nancy Diabetic ketoacidosis Chronic hypertension Pregnancy-induced hypertension	. 1 4 4		1 2 2		6 2 . 11		8 9	
Total hospital days	14	13.2 ± 1.8	3	12.5 ± 2.5	48	17.3 ± 1.7	57	12.0 ± 0.9

VUH = Vanderbilt University Hospital.

initial visit to Vanderbilt University Medical Cener were not significantly different among the groups. All infants in group 1 were delivered by cesarean section at the time a positive contraction stress test was cheserved. Six of the infants were subsequently observed to have major disorders. These disorders were congenital heart defects in two, myelomeningocele with hydrocephalus, syringomyelia, microcephaly, and hydrocephalus, syringomyelia, microcephaly, and hydrops. When these six infants are excluded, the nematal outcome of the infants noted to have fetal distress diagnosed by antepartum monitoring was not significantly worse than that of infants in other groups (Talle IV). The occurrence of major congenital anomalies was significantly greater in group 1 than in groups 2 and 3 combined (p < 0.01).

As previously stated, the indication for delivery n group I was a positive contraction stress test, which was thought to represent possible fetal distress. Indications for delivery of women in group 2 were mature lecithing sphingomyelin ratios in 18, presentation in active laborat term in 12, preeclampsia/eclampsia in 11, hydrannios in five, presentation in preterm labor and premature delivery in two, presentation with premature

rupture of the membranes in two, chronic hypertension in two, previous in utero fetal death in one, abruptio placentae in one, and bloody amniocentesis in one. In Group 3, the indications for delivery were mature lecithin/sphingomyelin ratios in 31, presentation at term in labor in 25, preeclampsia/eclampsia in nine, chronic hypertension in eight, presentation with premature rupture of the membranes in three, polyhydramnios in two, preterm labor in one, and Rh disease in one. (Some of the patients in groups 2 and 3 had more than one indication for delivery.)

When group 1A is compared with groups 2 and 3, the gestational age at delivery was earlier and the weight, head circumference, and length of the infants were less (Table IV). However, the Apgar scores of the infants in all three groups were not different. None of the infants in group 1A developed hyaline membrane disease, and the lengths of hospitalization for the infants in all three groups were not different.

When infants who had negative contraction stress tests (group 2) are compared with those who had reactive nonstress tests (group 3) throughout pregnancy, the former were delivered at an earlier gestational age

,		Significanc	e	
1 vs. 2	1 vs. 3	1A vs. 2	1A vs. 3	2 vs. 3
NS	NS	ŊS	NS	NS ·
NS	NS		."	NS

			3.00	
NS	NS	NS ·	NS	p < 0.01

(Table IV). Subsequently, the distribution of these infants between the nursery and the neonatal intensive care unit was also different. Furthermore, excluding those infants with congenital anomalies that could contribute to respiratory distress, the occurrence of respiratory disorders in general and hyaline membrane disease in particular was more common in group 2 than in group 3 (p < 0.01).

Comment

Historically, insulin-dependent diabetic pregnancies were at increased risk for unexplained intrauterine fetal deaths. Consequently, while the development of fetal pulmonary maturity is awaited in these pregnancies, antepartum fetal monitoring is conducted with the goal of minimizing unexplained stillbirths. During the 6year period described in this report, antepartum monitoring was associated with an absence of unexplained stillbirths among insulin-dependent pregnant diabetic patients delivered at our institution.

Antepartum fetal monitoring at Vanderbilt University Medical Center has continued to evolve over the 6year period of this report. In the initial year, 1977, many patients underwent daily urinary estriol measurements in addition to nonstress tests. This testing was subsequently curtailed because of (1) increasing familiarity with nonstress tests and reliance on them in the face of falling estriol levels in order to postpone delivery as long as possible, (2) the practical necessity of patient hospitalization for performance of this test, and (3) difficulty in accurate collection of urine and measurement of estriol levels. Currently, measurements of estriol levels are not used at all. Furthermore, increasing use of outpatient nonstress tests in subsequent years was ir. keeping with our general transition from inpatient to outpatient management of insulindependent diabetic pregnancies.2 Twice-weekly nonstress tests were initiated in 1981 on the basis of a review of the .000 nonstress tests at Vanderbilt University Medical Center that identified pregnant diabetic patients as a high-risk group.10 The final change was the introduction of the biophysical profiles in 1982 in an occasional patient. The role of the biophysical profile in our management of diabetic pregnancies is still developing, and an absolute protocol for its use has not been established.

The use of nonstress tests, oxytocin challenge tests, and biophysical profiles is not new to obstetrics in general^{6.8} or diabetic pregnancies in particular.^{2,3,5,11,12} Use of estriol levels in conjunction with contraction stress tests3. 5. 11 and nonstress tests5. 11 has been demonstrated to be reliable as an indication of fetal wellbeing. However, in all of these reports, estriol levels were, at least in part, relied on.

Recently, Golde et al.12 reviewed their experience with 107 consecutive pregnant diabetic patients in whom the primary antepartum fetal surveillance method was twice-weekly nonstress tests followed up by contraction stress tests and biophysical profiles. Among 55 patients in their series with nonreactive nonstress tests only one did not have a reassuring followup test. This patient was delivered of her infant and did not suffer major morbidity. However, because only one infant had fetal distress, the sensitivity of the screening protocol had only limited assessment.

Our findings, in which the number of infants noted to have positive contraction stress tests was increased, confirm the efficacy of this screening protocol. Furthermore, when we exclude infants that had major disorders and would have had major illnesses regardless of when they were delivered, these findings also confirm the potentially healthy outcome for infants of insulin-dependent diabetic pregnancies. The eight infants in group IA had Apgar scores similar to those of infants in groups 2 and 3, required similar subsequent lengths of hospitalization, and did not suffer from hyaline membrane disease. The good outcome in association with prompt action for fetuses with positive contraction seress tests should not be unexpected, since

Table IV. Neonatal outcome following antepartam monitoring of insulin-dependent diabetic pregnancies

		Positive corere	-cEon str	ress test	Group 2: Nonreactive nonstress test, negative contraction stress test (n = 48)			oup 3: Reactive ress test $(n = 57)$
	Gra	$up \ 1 \ (n = 14)$	Gro	$up\ 1A\ (n=8)$				
	No.	Mean ± SEN	No.	Mean ± SEM	No.	Mean ± SEM	No.	Mean ± SEM
Estimated gestational age at delivery (wk)	12	35.3 ± 0.7	8	35.3 ± 0.9	44	36.6 ± 0.4	51	37.8 ± 0.3
Nursery Neonatal intensive care unit	2 12		2 6		14 34		33 24	
Apgar scores 1 min 5 min	14 14	5.6 ± 0.7 7.7 ± 0.5	. 8	6.9 ± 0.9 8.4 ± 0.5	47. 45	7.3 ± 0.2 8.4 ± 0.2	56 54	7.1 ± 0.2 8.5 ± 0.1
Infant weight (gm) Head circumference (cm) Length (cm)	14 13 13	2401 ± 21C 31.8 ± 1.2 44.2 ± 1.4	8 7 7	2282 ± 235 31.3 ± 1.2 44.0 ± 1.7	48 43 42	3272 ± 110 34.3 ± 0.3 48.9 ± 0.7	57 47 46	3402 ± 94 34.8 ± 0.6 49.7 ± 0.7
Respiratory disorders Hyaline membrane disease	9 1		4		16 8		6 2	
Respiratory distress syndrome type II	3	;	.1		1 ;		1	•
Pulmonary edema Aspiration	3 2	á	3	•	7	•	4	
Congenital abnormalities None Cardiac Myelomeningocele Hydrocephalus Syringomyelia	8 2 1 1		8		46 1		53 1 1 1	• ,
Single umbilical artery Microcephaly	1				1		1	
Delivery complications Shoulder dystocia Fetal fracture Fetal nerve injury	٠	·		· •	1		3 2 1	
Length of neonatal hospitalization (days)	12	9.2 ± 2.€	. 8	7.5 ± 1.8	46	7.2 ± 0.6	57	6.1 ± 0.5

^{*}Analysis excludes respiratory disorders in infant: with congenital anomalies contributing to respiratory distress.

previous reports have indicated repeatedly that such action will provide a good outcome. If Furthermore, it is probably correct to surmise that these infants with fetal distress diagnosed at routine antepartum monitoring would have died in an earlier era. Thus it is these infants that historically would have been 'unexplained stillbirths."

It is interesting to note that none of the infacts of diabetic mothers delivered because of the antepartum identification of fetal distress in our series and have in other series. 11.12 suffered from hyaline membrane disease despite the early gestational age at delivery. This implies that the stress these fetuses experience may in some way potentiate more rapid development of ung maturity than that which normally occurs in diabetic pregnancies. With regard to the outcomes of pregnancies in groups 2 and 3, the former tended to be feliv

ered at an earlier gestational age and to have a higher incidence of pulmonary disease, including hyaline membrane disease, and an increased need for the neonatal intensive care unit.

The occurrence of major congenital anomalies among insulin-dependent diabetic pregnancies noted to have fetal distress during antepartum fetal monitoring was statistically significant when compared with the occurrence in the remainder of the diabetic population. This association of abnormal fetal heart rate patterns with congenital anomalies has been previously described by Navot et al., ¹⁴ Rayburn and Barr, ¹⁵ and Phillips and Towell. ¹⁶ While antepartum monitoring should by no means be construed as an appropriate screening test for congenital anomalies, the identification of anomalies in association with the antepartum identification of fetal distress should be considered in

		,	Significar	ıce	
	1 vs. 2	1 vs. 3	IA vs. 2	IA vs. 3	2 vs. 3
			NS	p < 0.01	p < 0.02
	NS	p = 0.009			p = 0.0059
			NS	NS.	NS
			NS	NS	NS
			p < 0.001 p < 0.01 p < 0.02	p < 0.001 p < 0.05 p < 0.01	NS NS NS
	NS	p = 0.051* NS			p = 0.009* p = 0.041
_				p = 0.035	

NS NS NS

order that a patient not undergo an emergency cesarean section for a fetus with an abnormality incompatible with life.

In summary, this report has demonstrated the efficacy of antepartum electronic fetal monitoring in eliminating unexplained stillbirths in insulin-dependent diabetic pregnancies. Furthermore, it has shown that in infants without major disorders, identification and

timely delivery cf fetuses with antepartum distress resulted in the prevention of major neonatal morbidity.

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Amniotic fluid insulin, C peptide concentrations, and fetal morbidity in infants of diabetic mothers

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Glucose, insulin, C peptide, and insulin antibody concentrations were measured in amnictic fluid collected under basal conditions and 2 hours after an arginine challer ge from 61 insulin-treated diabetic women (12 basal and 49 after arginine challenge) and 31 nondiabetic x-squart women in late gestation (23 basal and eight after arginine challenge). The insulin, C peptide, and Loose concentrations were significantly higher in diabetic pregnant women than in nondiabetic pregnant vomen in each case. In the amniotic fluid obtained after arginine challenge in diabetic pregnant womer, C peptide concentration was correlated with both insulin concentration (r = 0.61) and birth weight (r = 1.53). The insulin and C peptide concentrations were significantly higher (p < 0.025) in samples from diabetc pregnancies associated with fetal morbidity than from diabetic pregnancies without fetal morbidity. Basa amniotic fluid insulin and C peptide concentrations were slightly greater in overweight infants of placetic mothers compared to those of normal weight, whereas the differences for insulin and C peptide car centrations in the amniotic fluid obtained after arginine challenge were highly significant (p < 0.0125 and z < 0.0005, respectively). Finally insulin and C peptide concentrations in the amniotic fluid obtained after aginine challenge in diabetic pregnant women showed a correlation with maternal metabolic control but no with the degree (White classification) of maternal diabetes. No or negligible interference of insulin at body in the radioimmunoassay of insulin in amniotic fluid was observed. (Am J OBSTET GYNECOL 1985; #E3:534-40.)

Key words: Amniotic fluid, insulin, C peptide, argi rine, diabetic pregnancy, fetal morbidity

For many years the knowledge of the composition of amniotic fluid was limited by the lack of amniotic fluid samples; more recently, because of the widespread use of amniocentesis, research into amniotic fluid composition has proliferated and many protein hormores have been found. In pregnancies that are complicated by conditions considered dangerous to the fetus, such as diabetes mellitus, changes in the amniotic fluid levels of some hormones may have a physiopathologic significance and perhaps a prognostic value.

For this purpose the determination of amniotic fluinsulin concentration has been suggested as being good indicator of the status of the fetus in utero and of the newborn child in insulin-dependent diabetic pregnancies. Current assessments of the fetal origin of amniotic fluid insuling suggest that insuling excreted in the fetal urine. However, the transplacental transfer

of maternal insulin-binding antibodies generated in insulin-treated diabetic mothers might cast some doubt on the significance of amniotic fluid insulin concentrations, since circulating fetal insulin may be bound, at least in part, by these antibodies.³

More recently, the ability to measure plasma C peptide, which is secreted from the pancreatic beta cell in equimolar concentrations with insulin, has provided a means to assess beta cell function despite the presence of circulating insulin antibodies. A similar assay has been applied to the study of infants of diabetic mothers. and, to a lesser extent, to amniotic fluid. 9-11

The possibility of evaluating insulin and C peptide in the amniotic fluid during diabetic pregnancy prompted us to investigate the concentration of these peptides in amniotic fluid that had been collected under basal conditions and 2 hours after an arginine challenge in the mother. Arginine was used because this aminoacid crosses the placenta⁸ and may stimulate fetal beta cells. If this reaction occurs, fetal hormonal responses in the amniotic fluid can be measured, thus enabling a derangement of insulin secretion to be identified.

Moreover, insulin antibodies were evaluated in both amniotic fluid and the blood of diabetic mothers in order to preclude their interference in insulin and C peptide radioimmunoassays.

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Table I. Personal and clinical data of the diabetic and nondiabetic pregnant women

<i>.</i>		A on Anni	Gestational age	C	Maternal contro	
Subjects	No.	Age (yr) (mean ± SEM)	at delivery (wk)	Cesarean section (%)	Good	Poor
Nondiabetic	41	28 ± 3	40 ± 0.4	. 9		_
Diabetic	٠,	-		``.		
Class A	23	32 ± 6.2	39 ± 0.2	33	74	28
Class B	22	34 ± 4.7	37 ± 0.2	57	55	45
Class C to F	16	28 ± 5.8	38 ± 0.2	73	31	45 69
Total	61 .	31 ± 5.2	38 ± 0.2	52	56	44

Table II. Neonatal complications in diabetic and nondiabetic pregnant women (2000 subjects)

Subjects	No.	Macrosomia (≥90th percentile) (%)	Hypoglycemia (≥25 mg/dl) (%)	RDS (%)	Hypocalcemia (≥7 mg/dl) (%)	Hyperbilirubinemic (≥10 mg/dl) (%)
Normal Diabetic	2000	12	0.2,	1.8	2.7	16
Class A	23	26.0	4.3	8.7	17.4	39.1
Class B	22	68.2	31.8	. 22.7	18.2	59.1
Class C to F	16	25.0	43.7	18.7	25.0	.68.7
Total	61	40.9	24.6	16.4	19.7	54.1

RDS = Respiratory distress syndrome.

Table III. Glucose, insulin, and C peptide concentrations (mean ± SEM) in amniotic fluid collected under basal conditions and 2 hours after arginine test from diabetic and nondiabetic women

:	Ва	sal amniotic flu	id	Arginine-	Statistical differences,		
Glucose Subjects (mg/dl)		Insulin (μU/ml)	C peptide (pmol/L)	Glucose (mg/dl)	Insulin (µU/ml)	C peptide (pmol/L)	basal versus arginine-stimulcted amniotic fluid
Normal ·	11 ± 2 (n = 23)	6 ± 1 $(n = 22)$	174 ± 45 (n = 10)	10 ± 1 (n = 8)	7 ± 1 $(n = 8)$	161 ± 41 (n = 8)	NS
Diabetic	38 ± 5 (n = 12)	11 ± 1 $(n = 12)$	357 ± 68 (n = 12)	49 ± 4 (n = 49)	28 ± 6 (n = 49)	717 ± 91 (n = 49)	p < 0.05*
Normal vs. diabetic	p < 0.0005	p < 0.005	p < 0.025	p < 0.0005	NS	$\dot{\mathbf{p}} < 0.0\dot{1}$	

^{*}C peptide concentrations.

Finally, the purpose of the present study was to investigate the relationship of amniotic fluid insulin and C peptide levels with the degree of maternal metabolic control and with the outcome of pregnancy.

Material and methods

Subjects. One hundred two amniotic fluid samples were obtained from 61 insulin-treated diabetic patients and from 41 nondiabetic control women.

Amniocenteses were required in all cases in order to determine fetal pulmonary maturity prior to an induction of labor or a repeat cesarean section. Insulindependent diabetic patients were admitted to the antepartum ward of the hospital at 32 to 35 weeks of gestation to ensure the optimal control of diabetes. The goal of treatment was to maintain the fasting plasma

glucose level <90 mg/dl (5 mmol/L) and the 2-hour postprandial plasma glucose level below 130 mg/dl (7.2 mmol/L).

Of 61 diabetic patients, 23 belonged to Class A, 22 to Class B, and 16 to Class C according to the White¹² classification. All diabetic women, irrespective of the White classification, were treated with insulin (some with commercial and some with highly purified insulins).

After delivery, all infants were clinically evaluated by experienced pediatricians and the gestational age was assessed according to Dubowitz' criteria.¹³

The infants from both diabetic and nondiabetic mothers were assessed as small for gestational age (less than the tenth percentile), appropriate for gestational age (tenth to ninetieth percentile), or large for gesta-

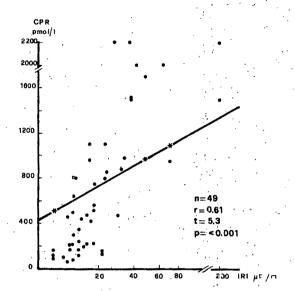


Fig. 1. Correlation between C peptide (CPR) and insuln (RI) concentrations in the amniotic fluid collected after an eg n ne challenge in 49 diabetic pregnant women.

tional age or macrosomic (greater than the nine in the percentile), according to the weight-gestational age classification of Lubchenco et al. 14

Hypoglycemia was diagnosed when blood gumse levels were recorded at values <25 mg/dl (1.4 mmcFL) 2 hours after birth; hyperbilirubinemia was diagnosed when bilirubin levels were >10 mg/dl; hypocameria was diagnosed when plasma calcium levels were <7 mg/dl. Signs of respiratory distress syndrome were locked for during the first 48 hours of life. The diagnose of respiratory distress syndrome was based on the scaplished clinical symptoms, blood gas values, and chest x-ray findings. Respiratory distress syndrome diagnosed according to the above criteria was mild in all but me case. The clinical parameters of diabetic and romainabetic women are summarized in Tables I and I.

Procedure. Amniotic fluid was collected from the diabetic women between 34 and 36 weeks of gestation and from the nondiabetic control subjects between 35 and 39 weeks of gestation.

Samples of amniotic fluid were obtained under betall conditions from 12 diabetic women (five Class A, three Class B, and four Classes C to F) and from 23 mordiabetic women. In 49 diabetic women (18 Class A, 18 Class B, and 13 from Classes C to F) and eight nordiabetic women, amniocentesis was performed 2 Lours after an arginine tolerance test (30-minute infusion of 30 gm of arginine monochloride dissolved in 400 mil of water).

All amniotic fluid samples were collected in upes containing ethylenediaminetetraacetic acid plus Trassylol (1.2 mg of ethylenediaminetetraacetic acid 500 mIU/ml) and then centrifuged at 4° C and frozen at -20° C until analysis.

Table IV. Glucose, insulin, and C peptide concentrations in amniotic fluid collected after arginine test ir diabetic pregnancy with and without fetal morbidity

Neonatal complication	Glucose (mg/dl)	Insulin (µU/ml)	C peptide (pmol/L)
Macrosomia			,
With	46 ± 2	46 ± 11	1135 ± 142
* * * * * * * * * * * * * * * * * * *	(n = 22)	(n = 22)	(n = 22)
Without	50 ± 7	13 ± 1	376 ± 66
	(n = 27)	(n = 27)	(n = 27)
	NS	p < 0.0125	p < 0.0005
Hypoglycemia		•	•
With	57 ± 9	48 ± 19	854 ± 185
	(n' = 13)	(n = 13)	(n = 13)
Without	45 ± 4	20 ± 3	667 ± 104
-	(n' = 36)	(n = 36)	(n = 36)
. *	· NS	p < 0.025	NS
Hypocalcemia		•	
With	58 ± 10	$52 \pm .22$	843 ± 208
	(n = 11)	(n = 11)	(n = 11)
Without	46 ± 4	21 ± 3	680 ± 101
	(n = 38)	(n = 38).	(n = 38)
and the second	NS	p < 0.01	NS -

The following were measured in each sample: glucose by a glucose oxidase method (Boehringer Glucose Analyzer), insulin as previously described,⁸ and C peptide according to the method described by Heding et al.⁶ (Novo's Lit was used for the C peptide radio-immunoassay).

Insulin antibodies, as insulin-binding capacity, were assayed as previously described, both in maternal blood (late gestation) and in the amniotic fluid of diabetic pregnant vomen.

In order to perform the statistical analysis, the levels of amniotic fluic glucose, insulin, and C peptide were compared in both the diabetic and the nondiabetic subjects.

The levels of amniotic fluid insulin and C peptide found under basal conditions and after arginine challenge were also compared in infants of diabetic mothers, with and without complications, between classes of maternal diabetes and between groups with different degrees of metabolic control.

Maternal metabolic control was evaluated on the basis of blood glucose levels measured three times monthly, glycosuria assessed four times daily, and the occurrence of hypoglycemia and/or acetonuria. Two degrees of maternal metabolic control were arbitrarily set, to facilitate statistical comparison. Good maternal metabolic control was diagnosed when severe hypoglycemia or acetonuria was absent throughout the entire course of pregnancy, blood glucose levels were never >160 mg/dl, and glycosuria was a rare event. Poor maternal metabolic control was diagnosed when severe hypoglycemia or acetonuria occurred occasionally, repeated

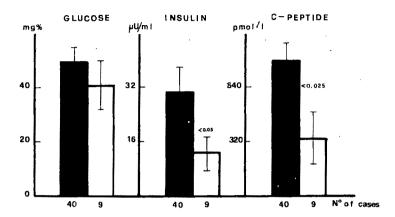


Fig. 2. Glucose, insulin, and C peptide concentrations in amniotic fluid collected after an arginine challenge in 49 diabetic pregnancies with (dark columns, and without (light columns) fetal morbidity.

Table V. Glucose, insulin, and C peptide concentrations in amniotic fluid collected after arginine test in relation to degree of maternal diabetes by White classification

White class	No.	Glucose (mg!dl)	Insulin (μU/ml)	C peptide (pmol/L)
A	18	36 ± 4*	27 ± 11	620 ± 182 850 ± 186 665 ± 158
B	18	50 ± 7†	35 ± 11	
C to F	13	64 ± 9*	19 ± 4	

*Statistical difference: p < 0.0025. †Statistical difference: p < 0.05.

blood glucose levels were frequently >160 mg/dl, and glycosuria was often present.

Results

Insulin antibodies. There were no insulin antibodies in the blood (<0.05 mU/ml, group 1) in 26 of 61 diabetic women (15 Class A, eight Class B, and three Classes C to F); insulin antibody levels were medium to low (>0.05 to 2.0 mU/ml, group 2) in 30 of 61 diabetic women (six Class A, 12 Class B, and 12 Classes C to F) and high (>2.0 mU/ml, group 3) in the other five diabetic women (two Class A, two Class B, and one Classes C to F). Amniotic fluid insulin antibodies were absent in all but three cases: 0.09 mU/ml in a Class A woman (corresponding blood insulin antibodies were absent) and 0.26 mU/ml (corresponding blood insulin antibody level was 0.24 mU/ml), and 0.31 mU/ml (blood insulin antibody value was 0.38 mU/ml) in two Class C to F women. In these three cases insulin and C peptide concentrations were 11, 4, and 12 µU/ml and 800, 100, and 350 pmol/L, respectively.

Insulin and C peptide concentrations in amniotic fluid collected under basal conditions (diabetic versus control women). As shown in Table III, glucose, insulin, and C peptide concentrations were significantly higher in the diabetic women than in the control women.

Insulin and C peptide concentrations in amniotic fluid collected 2 hours after arginine infusion (diabetic versus control women). As shown in Table III, g ucose, insulin, and C peptide concentrations were even more elevated in the diabetic women than in the control women and the differences in glucose and C peptide concentrations were significantly greater than those under basal conditions.

Effect of arginine. As shown in Table III, glucose, insulin, and C peptide concentrations in the basal and in arginine-stimulated amniotic fluid were very similar in control women; in contrast insulin and C peptide values in arginine-stimulated amniotic fluid were greater than those found in basal amniotic fluid, with the difference in C peptide levels in the diabetic women being significant.

The analysis of insulin and C peptide concentrations found in the arginine-stimulated amniotic fluid showed that there was a significant correlation between the two peptides in the diabetic women (r = 0.61, p < 0.001) (see Fig. 1).

Insulin and C peptide concentrations in argininestimulated amniotic fluid and in amniotic fluid associated with fetal morbidity in diabetic pregnancy. Insulin and C peptide concentrations were significantly higher in the amniotic fluid of infants of diabetic mothers with fetal morbidity than in those without (see Fig.

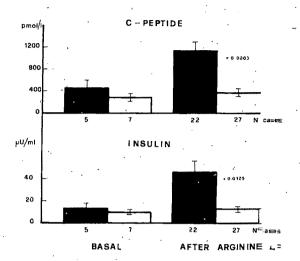


Fig. 3. Insulin and C peptide concentrations in amnior.c —uid collected under basal conditions (on the left) and after an arginine challenge (on the right) from 61 diabetic pregnancies with delivery of overweight (dark columns) and normal weight (light columns) infants.

2). In particular, insulin concentrations were $\leq g$ filtrantly higher in the amniotic fluid of macrocomic (p < 0.125), hypoglycemic (p < 0.0025), and hypocalcemic (p < 0.01) infants of diabetic mothers than in the amniotic fluid of infants of diabetic mothers without these complications. C peptide concentrations vere also significantly higher in the amniotic fluid of infants of diabetic mothers with macrosomia (see Table IV)

Amniotic fluid insulin and C peptide and macrosomia. Insulin and C peptide concentrations were slightly but not significantly greater in the basel anniotic fluid of macrosomic infants of diabetic methers than in that of infants of diabetic mothers of normal weight, whereas the differences in insulin and C peptide concentrations in the arginine-stimulated amnittic fluid between overweight infants of diabetic methers and those of normal weight were highly significant see Fig. 3).

A significant linear correlation between birth verifies and C peptide concentrations was also observed in the arginine-stimulated amniotic fluid (r = 0.53, p < 0.1.) (see Fig. 4). The prognostic value of insulin and C peptide concentrations in the arginine-stimulated anniotic fluid was also evaluated in relation to the main value of the two peptides. We observed that 92.6% of the overweight infants of diabetic mothers and 7:-% of those of normal weight had insulin values that water above the mean (28 + 6 μ U/ml) and that 75% of the overweight infants of diabetic mothers and 25% of those of normal weight had C peptide values that water above the mean (717 + 91 pmol/L), the difference in values between overweight and normal weight infants being highly significant in any case (p < 0.001).

Insulin and C peptide concentrations in arginize-

stimulated amniotic fluid in relation to degree of maternal diabetes mellitus evaluated according to White classification. As shown in Table V glucose concentrations became progressively higher in Classes A, B, and C to F. Insulin and C peptide concentrations did not differ statistically between the three groups.

Insulin and C peptide concentrations in the arginine-stimulated amniotic fluid in relation to maternal metabolic control. As shown in Table VI, there were slightly higher glucose concentrations and significantly higher insulin and C peptide concentrations in the amniotic fluid of patients with poor maternal metabolic control compared to those found in the amniotic fluid of patients with good maternal metabolic control.

Comment

This study showed that insulin and C peptide concentrations in the amniotic fluid were correlated and that the interference of insulin antibodies in the radio-immunoassay of insulin and C peptide in the amniotic fluid, even if present, was negligible. Moreover, it demonstrated that an arginine infusion in a diabetic pregnant woman can amplify the derangement of beta cell function in the fetus. It is important to note that arginine, unlike insulin, crosses the placenta and for this reason it is likely that the addition of arginine could induce a further increase of insulin secretion in many fetuses of diabetic mothers that are already exposed to maternal hyperglycemia.

This study also demonstrated that insulin and C peptide levels in the amniotic fluid collected after arginine were correlated with an increased risk of fetal macrosomia, hypoglycemia, and hypocalcemia and in general with fetal morbidity. They were also correlated with maternal metabolic control rather than with the degree of maternal diabetes according to the White classification.

Interestingly amniotic fluid insulin and C peptide appeared to be more sensitive parameters than amniotic fluid glucose in distinguishing complicated from noncomplicated diabetic pregnancies. Our results confirm that amniotic fluid insulin and C peptide concentrations are a reliable indicator of fetal prognosis in diabetic pregnancies and that a high level of amniotic fluid insulin or C peptide should alert both obstetricians and pediatricians to the potential risk of macrosomia and hypoglycemia as well as hypocalcemia.

Some discrepancies exist in the literature about the reliability of amniotic fluid insulin and C peptide as prognostic indicators of fetal outcome. ¹⁵ According to some authors ^{15, 16} amniotic fluid C peptide seems more reliable than amniotic insulin. On the basis of our results, we cannot support this conclusion. On the contrary, we have observed a linear correlation between amniotic fluid insulin and C peptide concentrations.

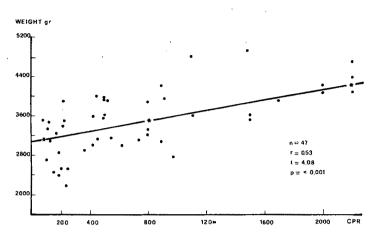


Fig. 4. Correlations between C peptide (CPR) concentrations in amniotic fluid collected after an arginine challenge and birth weight in diabetic pregnancy.

Table VI. Glucose, insulin, and C peptide concentrations in amniotic fluid collected after arginine test in relation to maternal metabolic control during pregnancy

Maternal metabolic control	No.	Gluco⊊ (mg!d₁)	Insulin (μU/ml)	C peptide (pmol/L)
Good	27	47 ± 5	17 ± 3	535 ± 91
Poor	22	51 ± 7	41 ± 11	940 ± 158
Statistical difference		NS	p < 0.025	p < 0.0125

The amniotic C peptide and insulin levels are also significantly correlated with fetal morbidity and maternal metabolic control. It is likely that the new model used here (that is, the collection of amniotic fluid after arginine) may have eliminated some of the differences found by other authors.

In particular, a clear correlation between amniotic fluid insulin and C peptide concentrations has been observed in the amniotic fluid of macrosomic infants of diabetic mothers where the two peptides showed the same prognostic value.

High levels of insulin and C peptide in amniotic fluid suggest the possibility that hyperplasia or hyperactivity of fetal islet beta cells is promoting an excessive secretion of endogenous insulin. This excessive secretion may be due either to the degree of maternal diabetes according to the White classification or to poorly controlled maternal diabetes. Our findings suggest that impaired maternal metabolic control plays a prominent role during pregnancy. In this context it is worth noting that as many as 13 of 22 diabetic women from White Class A or B had poor maternal metabolic control. This observation shows how easily the subjects in these two classes (most of them were not insulin dependent when not pregnant) may be treated either inappropriately or too late. Therefore, in the management of diabetic pregnancies an optimal metabolic control throughout pregnancy is the most important factor in decreasing perinatal mortality and morbidity. Indeed the tight con-

trol of maternal blood glucose levels has already been reported to be associated with a low perinatal mortality rate in diabetic pregnancy. 15, 16 However, it is also important to underline the fact that neonatal complications, such as hypocalcemia and hypoglycemia, associared with higher amniotic fluid insulin and C peptide levels can be related to a beta cell hyperfunction even in normal weight infants of diabetic mothers.

Finally it should be noted that even the lowest values of amniotic fluid insulin and C peptide of those infants of diabetic mothers with no neonatal complications were about twice those observed in the amniotic fluid cf normal infants. This suggests that during diabetic pregnancy the fetal beta cell hyperfunction is possibly cue to other causes in addition to the poor maternal metabolic control.

In conclusion the measurements of amniotic fluid insulin and C peptide after arginine stimulation have proved to be useful for the prediction of fetal outcome in diabetic pregnancy. Further studies are needed before broad clinical implications can be accurately def.ned. Nevertheless the assessment of insulin and C peptide in the amniotic fluid during pregnancy can reveal valuable physiopathologic aspects and provide a useful prenatal diagnostic technique for monitoring the impact of maternal diabetes on the fetus. Both can help devise the most effective plan of diabetic management and may lead to a further reduction in perinatal mortality and morbidity in diabetic pregnancies.

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Decreased levels of amniotic fluid α -fetoprotein associated with Down syndrome

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Low maternal serum α -fetoprotein levels have been associated with fetal aneuploidies. Amniotic fluid α -fetoprotein levels have been reported to be low with Down syndrome (trisomy 21) but not with other fetal trisomies. We compared the amniotic fluid α -fetoprotein levels from 25 cases of autosomal trisomy (18 of trisomy 21, four of trisomy 13, three of trisomy 18) diagnosed by midtrimester fetal cytogenetic studies with those from matched, cytogenetically normal pregnancies. With these normal pregnancies used as controls, statistical analyses were performed on the data for all the trisomic fetuses, on the data for trisomy 21 only, and on the data for trisomies 13 and 18 combined. Amniotic fluid α -fetoprotein levels were significantly lower in the 25 trisomic cases compared with controls, 0.77 \pm 0.34 versus 1 03 \pm 0.34 mg/dl (p < 0.001). However, further analysis revealed that the difference was due to the trisomy 21 data alone. In the trisomy 21 cases there was a significant difference for α -fetoprotein levels between cases and controls (p < 0.001), whereas there was no difference for the combined trisomy 13 and 18 cases compared to controls (p > 0.40). These findings suggest that the low maternal serum levels of α -fetoprotein reported in cases of Down syndrome may be related to reduced amniotic fluid concentrations. However, the reduced maternal serum α -fetoprotein levels reportedly associated with trisomies 13 and 18 do not seem to be explained by low amniotic fluid concentrations. (AM J OBSTET GYNECOL 1985;153:541-4.)

Key words: Amniotic fluid α -fetoprotein, maternal serum α -fetoprotein, fetal aneuploidies

Increased levels of a-fetoprotein measured in maternal serum and amniotic fluid during the midtrimester of pregnancy are associated with fetal anomalies, especially open neural tube defects (anencephaly and spina bifida).1 Prenatal screening programs to measure midtrimester maternal serum α-fetoprotein levels have been established to identify those pregnancies at risk for neural tube defects. Diagnostic amniocentesis with measurement of amniotic fluid α-fetoprotein is recommended for those screened pregnancies found to have elevated maternal serum levels. An association has been reported between low levels of maternal serum αfetoprotein and chromosomal aneuploidies.^{2,3} More recently, Down syndrome (trisomy 21) has been associated with decreased \(\alpha \)-fetoprotein levels in both maternal serum and amniotic fluid.3,4 Decreased amniotic fluid α-fetoprotein concentrations in trisomic pregnancies could explain the reported association between low

levels of maternal serum α -fetoprotein and fetal trisomies. However, other studies have failed to establish a correlation between amniotic fluid and maternal serum α -fetoprotein levels, either in normal gestations or in those pregnancies with fetal chromosomal aneuploidy. We reviewed our cases of autosomal trisomies diagnosed by midtrimester fetal cytogenetic studies to determine whether amniotic fluid α -fetoprotein levels were decreased in these pregnancies when compared to those in a matched group of pregnancies with normal fetal cytogenetic studies.

Material and methods

The records of all autosomal trisomies that were diagnosed prenatally in the Laboratory of Medical Genetics at The University of Alabama at Birmingham from January, 1979, to September, 1984, were retrospectively reviewed. The prenatal diagnosis program at The University of Alabama at Birmingham is a cooperative effort between the Department of Obstetrics and Gynecology, which provides diagnostic ultrasound and amniocentesis services, and the Laboratory of Medical Genetics, which provides genetic counseling and laboratory services. Most of the midtrimester amniotic fluid samples that are cultured and karyotyped by this laboratory are obtained at our institution from patients

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Table I. Results in 25 pregnancies with an autosomal trisomy and 75 controls

Variable	Group	n	Mean	SD	p*	
Maternal age (yr)	Trisomy	25	38.4	3.7	NS	
Material age (yr)	Control	75	37.4	2.5	143	
Parity	Trisomy	25	2.7	2.9	NIC	
· uin,	Control '	75	1.8	1.2	NS	
Gestational age (days)	Trisomy	25	119.3	7.4	NS	
Ocstational age (days)	Control 75		116.8	5.9	149	
Biparietal diameter (mm)	Trisomy	25	36	0.42	NS	
Diparietai thameter (min)	Control	66	36	0.33	140	

^{*}Probability, based on t test.

referred for consultation. Each patient who undergues an amniocentesis for fetal karyotype is offered amn_otic fluid α-fetoprotein determination. Prior to each zmniocentesis, an ultrasound examination was performed to document fetal viability, to confirm gestational 12e, to detect multiple gestation, and to choose the am-incentesis site. Transplacental puncture was avoided whenever possible. The amniocentesis was performed with a 22-gauge spinal needle. The initial 2 or 3 md of amniotic fluid was discarded. From the 30 ml of amniotic fluid that was then removed, 2 ml was sent for α-fetoprotein determination. The remaining amniocic fluid was centrifuged and the cells initiated into culture. Cytogenetic studies were performed according to star!dard techniques. All α-fetoprotein samples were maï ed to the same reference laboratory (AM). All α-fetop=tein determinations were done by rocket electroanmunodiffusion, for which the normal ranges at each second-trimester week have been published.6

This study was restricted to singleton pregnances and only to those women who had amniocentesis performed at our institution. Each case of trisomy vas matched with three controls, each of which had vield a normal fetal karyotype from nonbloody amniocid fluid. The trisomies and controls were matched fr race, fetal sex, gestational age (within 1 week), materral age (within 5 years). Each control chosen was from 3 woman who had amniocentesis in close temporal prosimity to the amniocentesis that had disclosed the trail somic fetus. Adequate follow-up data were available for all controls. Other variables that were tabulated included parity, biparietal diameter determined by ultrasound, placental localization, the number of needle insertions at the time of amniocentesis, and the physician: who performed the amniocentesis. Gestational age at time of delivery and birth weight were examined in the control group. Gestational age was defined by last merstrual period unless there was a 10-day or greater di - : crepancy in the estimated gestational age by fetal bparietal diameter obtained during the ultrasound examination. In cases of such a discrepancy the gestational age defined by ultrasound was used. Amniocenteses were performed between 15½ and 18 gestational weeks, so defined.

Results are reported as the means and standard deviation of the means. Univariate tests were made on the data with either contingency tables or t tests used as appropriate for categorical or measured responses. These tests were done in order to ascertain that the two groups were comparable for variables other than the one of primary interest (α-fetoprotein). An analysis of variance was run to test the equality of the mean αfetoprotein response for the two groups after the effects of matching were blocked out (repeated measures analysis of variance). The correlation of α -fetoprotein with the ultrasound parameters was assessed with Pearson correlation coefficients. The above analyses were run for all the data collected, on the data for trisomy 21 only, and on the combined data for trisomies 13 and 18. A probability level < 0.05 was considered significant.

Reculte

A total of 36 cases of autosomal trisomy were diagnosed during the study period. Ten cases were excluded, either because the samples of fluid were sent in from outside offices (three trisomy 21, two trisomy 18), because no α-fetoprotein determination was done (one trisomy 18, one trisomy 13), or because the fetus had an omphalocele (two trisomy 18, one trisomy 13), which is known to be associated with an elevated amniotic fluid α-fetoprotein concentration.⁷ Another case of trisomy 18 was excluded because of advanced gestational age, for which no appropriate controls were available. Therefore, 25 pregnancies with an autosomal trisomy (18 trisomy 21, four trisomy 13, three trisomy 18) were compared with 75 controls. All the Down syndrome cases were trisomy 21 except one, which was a translocation. For each of the 100 patients, only one amniotic fluid sample was needed for successful karyotyping and α-fetoprotein determination.

Analysis of the outcome of the 75 control pregnancies revealed a mean gestational age of 278 ± 11 days at

Table II. Mean α -fetoprotein levels by group

Group	n	Mean	SD	Range	<i>p</i> *
Trisomy	25	0.77	0.54	0.29-1.90	<0.001
Control ·	75	1.03	0.54	0.52-2.44	V0.001
Trisomy 21	18	0.70	0.24	0.29-1.09	< 0.001
Control	54	1.02	0.27	0.52-1.56	10.003
Trisomies 13 and 18	7	0.95	0.52	0.41-1.90	>0.40
Control	21	1.08	0.49	0.55-2.44	- 0.10

^{*}Probability, based on analysis of variance.

delivery. There were two preterm births, both occurring between 36 and 37 weeks. The average birth weight was 3445 ± 467 gm. Two babies weighed <2500 gm (2041 and 2098 gm). Six babies weighed >4000 gm, the largest weighing 4564 gm. On the basis of written follow-up reports from the referring physicians, none of these 75 babies was reported to have a congenital anomaly.

There were no differences between the trisomy and control groups with regard to maternal age, parity, gestational age at time of amniocentesis, or ultrasound biparietal diameter (Table I). Parity tended to be higher in the trisomy group but this did not reach statistical significance (p = 0.15). Of the amniocenteses, 84% from the control group and 90% from the trisomy group were done by one of the authors (R. O. D.). Three of the trisomies occurred in black patients, and the remainder in white ones. Eleven fetuses in the trisomy group were female, and 14 were male. Thirtythree control fetuses were female and 42 were male. Amniotic fluid α-fetoprotein levels were negatively correlated with biparietal diameter (r = 0.55, p < 0.001). Placental location (anterior versus posterior or fundal) had no effect on α -fetoprotein levels (p > 0.40). There was no difference between groups in the number of amniocentesis attempts necessary; only one needle insertion was required for 91% of the trisomy group and for 96% of the control group (p = 0.39).

Amniotic fluid α-fetoprotein levels were significantly lower in the trisomy group compared with controls, 0.77 ± 0.34 versus 1.03 ± 0.34 mg/dl (p < 0.001). Moreover, further analysis revealed that this difference was due to the trisomy 21 data. In the cases of trisomy 21, there was a significant difference for α -fetoprotein levels between the groups (p < 0.001), whereas there was no difference for the combined trisomy 13 and 18 data (p > 0.40) (Table II).

Comment

We found lower amniotic fluid α-fetoprotein levels in the group of Down syndrome fetuses than in matched controls but not in the combined group of fetuses with trisomy 13 or 18. The finding that amniotic fluid α-fetoprotein was negatively correlated with fetal biparietal diameter was anticipated, since amniotic fluid α-fetoprotein levels decrease with advancing gestational age.6 Other than the abnormal karyotype, there was no identified explanation for the decreased amniotic fluid levels found in association with a trisomy 21 fetus. These studies suggest that α -fetoprotein productior may be decreased in the fetus with Down syn-

The physiologic characteristics of α-fetoprotein have been extensively described. 1.8 Briefly, a-fetoprotein is synthesized initially by the yolk sac, then by the fetal gastrointestinal tract and liver. In the second trimester, the predominant site of production is the fetal liver. The fetal plasma level peaks between 10 and 13 weeks and then progressively declines until term. α-Fetoprotein is excreted in fetal urine and thus into amniotic fluid. Peak concentrations of amniotic fluid α-fetoprotein occur at 12 to 14 weeks' gestation. Conversely, the maternal serum α-fetoprotein levels rise steadily until 28 to 32 weeks of gestation. Since fetal serum and amniotic fluid α-fetoprotein levels are falling while maternal serum levels are rising, a simple diffusion mechanism from one compartment to another is unlikely. In fact, Barford et al.5 found no correlation between maternal serum and amniotic fluid α-fetoprotein at 16 to 17 veeks' gestation in normal pregnancies. However, in conditions of markedly elevated amniotic fluid αfetoprotein content, such as an encephaly or open spina bifida, maternal serum levels are markedly elevated.1 Screening programs for neural tube defects based on measurement of maternal serum α-fetoprotein levels are already under way in many areas and are currently being advocated and developed in others. 1.9-11

Recent articles have focused attention on the significance of markedly decreased serum α-fetoprotein levels in association with fetal chromosomal abnormalities.2,3 Merkatz et al.2 reported an association between decreased maternal serum \(\alpha \)-fetoprotein levels and fetal aneuploidies. They found all 12 maternal serum αfetoprotein samples associated with trisomy 13 (two cases) and trisomy 18 (10 cases) were below the established median for maternal serum α-fetoprotein. Maternal serum α-fetoprotein levels were below the median in 16 of 20 cases of trisomy 21. They fourd no association between amniotic fluid α-fetoprotein and any fetal chromosomal aneuploidy. However, Cackle et al.3.4 found lower levels of both amniotic fluic and maternal serum α-fetoprotein to be associated with Down syndrome. Subsequently, Nelson and Petersen¹² reported low levels of amniotic fluid α-fetoprote.n in their Down syndrome cases but not in other chramosomal aneuploidies. Our study thus supports the indings of Nelson and Petersen and suggests that the reported decreases in maternal serum α-fetoproteia associated with Down syndrome could be related to decreased fetal production of a-fetoprotein.

The mechanisms by which maternal serum α-Tetoprotein levels are reduced in a pregnancy with a trisomic fetus are not clear. Possible causes of low maternal serum α-fetoprotein in a trisomic pregnancy might include decreased production, increased degradation, alterations in molecular structure of α -fetoproteir, or decreased permeability of the trisomic placenta and membranes. Decreased fetal production in Down syndrome is suggested by our study and other prevous studies. However, to our knowledge, fetal serum afetoprotein in Down syndrome has not been reported. A mechanism other than decreased fetal production of α-fetoprotein is likely to be operative in trisomie: 13 and 18, as amniotic fluid levels seem to be normal. Using isoelectric focusing, ion exchange chroma ography, and agarose electrophoresis, Alpert et al.15 have described two molecular forms of a-fetoprotein. Aterations of molecular structure could lead to an altered rate of degradation or permeability across membranes. Alternatively, since the placenta and membranes are genetically abnormal, there may be changes in membrane permeability, so that fetal α-fetoprotein might not have normal access to the maternal compartment.

Although the mechanism remains controversial, decreased levels of maternal serum α -fetoprotein are clearly associated with fetal chromosomal abnormalties. On the basis of low levels of maternal serum α -fetoprotein, Baumgarten et al. found 4.9% of screened pregnancies in women less than age 35 to be at risk for chromosomal aneuploidies. The ratio for chromosomal aneuploidy in the group at risk was 1 in 63. The use of low maternal serum α -fetoprotein in women under age 35 has been estimated to identify

20% to 25% of Down syndrome pregnancies in this group.³ Therefore, obstetricians must be prepared to counsel, concerning an increased risk of chromosomal aneuploidy, their patients with a low serum α -fetoprotein level.

Although from a statistical point of view we failed to show low amniotic fluid α -fetoprotein levels to be associated with trisomies 13 and 18, our numbers were small. Interestingly, five of seven α -fetoprotein values in this group were below the mean for the controls. Nelson and Petersen, 12 studying 11 such cases without anterior abdominal wall defects, found the α -fetoprotein levels to be evenly distributed about the mean of the controls. Further studies correlating maternal serum, amniotic fluid, and fetal serum α -fetoprotein content and molecular structure should aid in the elucidation of the relationship between autosomal trisomy and α -fetoprotein.

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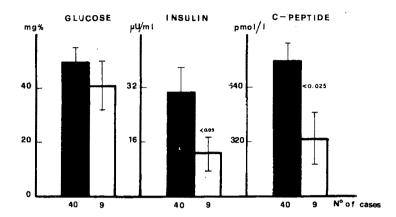


Fig. 2. Glucose, insulin, and C peptide concentrations in amniotic fluid collected after an arginine challenge in 49 diabetic pregnancies with (dark columns, and without (light columns) fetal morbidity.

Table V. Glucose, insulin, and C peptide concentrations in amniotic fluid collected after arginine test in relation to degree of maternal diabetes by White classification

White class	No.	Glucose (mg/dl)	Insulin (µU/ml)	C peptide (pmol/L)
A	18	36 ± 4*	27 ± 11	620 ± 182
В	18	$50 \pm 7 \dagger$	35 ± 11	850 ± 186
C to F	13	$64 \pm 9*$	19 ± 4	665 ± 158

*Statistical difference: p < 0.0025. †Statistical difference: p < 0.05.

blood glucose levels were frequently >160 mg/dl, and glycosuria was often present.

Results

Insulin antibodies. There were no insulin antibodies in the blood (<0.05 mU/ml, group 1) in 26 of 61 diabetic women (15 Class A, eight Class B, and three Classes C to F); insulin antibody levels were medium to low (>0.05 to 2.0 mU/ml, group 2) in 30 of 61 diabetic women (six Class A, 12 Class B, and 12 Classes C to F) and high (>2.0 mU/ml, group 3) in the other five diabetic women (two Class A, two Class B, and one Classes C to F). Amniotic fluid insulin antibodies were absent in all but three cases: 0.09 mU/ml in a Class A woman (corresponding blood insulin antibodies were absent) and 0.26 mU/ml (corresponding blood insulin antibody level was 0.24 mU/ml), and 0.31 mU/ml (blood insulin antibody value was 0.38 mU/ml) in two Class C to F women. In these three cases insulin and C peptide concentrations were 11, 4, and 12 µU/ml and 800, 100, and 350 pmol/L, respectively.

Insulin and C peptide concentrations in amniotic fluid collected under basal conditions (diabetic versus control women). As shown in Table III, glucose, insulin, and C peptide concentrations were significantly higher in the diabetic women than in the control women.

Insulin and C peptide concentrations in amniotic fluid collected 2 hours after arginine infusion (diabetic versus control women). As shown in Table III, glucose, insulin, and C peptide concentrations were even more elevated in the diabetic women than in the control women and the differences in glucose and C peptide concentrations were significantly greater than those under basal conditions.

Effect of arginine. As shown in Table III, glucose, insulin, and C peptide concentrations in the basal and in arginine-stimulated amniotic fluid were very similar in control women; in contrast insulin and C peptide values in arginine-stimulated amniotic fluid were greater than those found in basal amniotic fluid, with the difference in C peptide levels in the diabetic women being significant.

The analysis of insulin and C peptide concentrations found in the arginine-stimulated amniotic fluid showed that there was a significant correlation between the two peptides in the diabetic women (r = 0.61, p < 0.001) (see Fig. 1).

Insulin and C peptide concentrations in argininestimulated amniotic fluid and in amniotic fluid associated with fetal morbidity in diabetic pregnancy. Insulin and C peptide concentrations were significantly higher in the amniotic fluid of infants of diabetic mothers with fetal morbidity than in those without (see Fig.

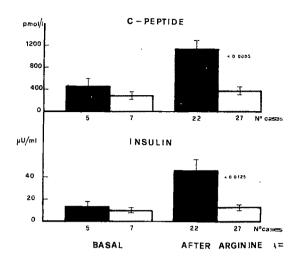


Fig. 3. Insulin and C peptide concentrations in amniotic fuid collected under basal conditions (on the left) and after an arginine challenge (on the right) from 61 diabetic pregnamies with delivery of overweight (dark columns) and normal weight (light columns) infants.

2). In particular, insulin concentrations were significantly higher in the amniotic fluid of macroscaric (p < 0.125), hypoglycemic (p < 0.0025), and hypocalcemic (p < 0.01) infants of diabetic mothers than in the amniotic fluid of infants of diabetic mothers without these complications. C peptide concentrations vere also significantly higher in the amniotic fluid of infants of diabetic mothers with macrosomia (see Table IV).

Amniotic fluid insulin and C peptide and macrosomia. Insulin and C peptide concentrations were slightly but not significantly greater in the basal imniotic fluid of macrosomic infants of diabetic mothers than in that of infants of diabetic mothers of normal weight, whereas the differences in insulin and C peptide concentrations in the arginine-stimulated amniptic fluid between overweight infants of diabetic mothers and those of normal weight were highly significant exerting. 3).

A significant linear correlation between birth weight and C peptide concentrations was also observed in the arginine-stimulated amniotic fluid (r = 0.53, p < 0.71) (see Fig. 4). The prognostic value of insulin and C peptide concentrations in the arginine-stimulated amniotic fluid was also evaluated in relation to the mean value of the two peptides. We observed that 92.6% of the overweight infants of diabetic mothers and 7.4% of those of normal weight had insulin values that were above the mean (28 + 6 µU/ml) and that 75% of the overweight infants of diabetic mothers and 25% of those of normal weight had C peptide values that were above the mean (717 + 91 pmol/L), the differences in values between overweight and normal weight infants being highly significant in any case (p < 0.001).

Insulin and C peptide concentrations in argining-

stimulated amniotic fluid in relation to degree of maternal diabetes mellitus evaluated according to White classification. As shown in Table V glucose concentrations became progressively higher in Classes A, B, and C to F. Insulin and C peptide concentrations did not differ statistically between the three groups.

Insulin and C peptide concentrations in the arginine-stimulated amniotic fluid in relation to maternal metabolic control. As shown in Table VI, there were slightly higher glucose concentrations and significantly higher insulin and C peptide concentrations in the amniotic fluid of patients with poor maternal metabolic control compared to those found in the amniotic fluid of patients with good maternal metabolic control.

Comment

This study showed that insulin and C peptide concentrations in the amniotic fluid were correlated and that the interference of insulin antibodies in the radio-immunoassay of insulin and C peptide in the amniotic fluid, even if present, was negligible. Moreover, it demonstrated that an arginine infusion in a diabetic pregnant woman can amplify the derangement of beta cell function in the fetus. It is important to note that arginine, unlike insulin, crosses the placenta and for this reason it is likely that the addition of arginine could induce a further increase of insulin secretion in many fetuses of diabetic mothers that are already exposed to maternal hyperglycemia.

This study also demonstrated that insulin and C peptide levels in the amniotic fluid collected after arginine were correlated with an increased risk of fetal macrosomia, hypoglycemia, and hypocalcemia and in general with fetal morbidity. They were also correlated with maternal metabolic control rather than with the degree of maternal diabetes according to the White classification.

Interestingly amniotic fluid insulin and C peptide appeared to be more sensitive parameters than amniotic fluid glucose in distinguishing complicated from noncomplicated diabetic pregnancies. Our results confirm that amniotic fluid insulin and C peptide concentrations are a reliable indicator of fetal prognosis in diabetic pregnancies and that a high level of amniotic fluid insulin or C peptide should alert both obstetricians and pediatricians to the potential risk of macrosomia and hypoglycemia as well as hypocalcemia.

Some discrepancies exist in the literature about the reliability of amniotic fluid insulin and C peptide as prognostic indicators of fetal outcome. ¹⁵ According to some authors ¹⁶ amniotic fluid C peptide seems more reliable than amniotic insulin. On the basis of our results, we cannot support this conclusion. On the contrary, we have observed a linear correlation between amniotic fluid insulin and C peptide concentrations.

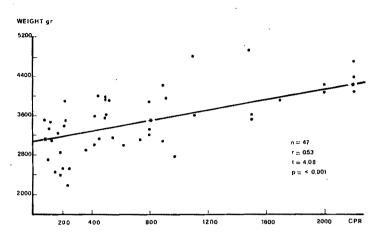


Fig. 4. Correlations between C peptide (CPR) concentrations in amniotic fluid collected after an arginine challenge and birth weight in diabetic pregnancy.

Table VI. Glucose, insulin, and C peptide concentrations in amniotic fluid collected after arginine test in relation to maternal metabolic control during pregnancy

Maternal metabolic control	No.	Glucose (mg/dl)	Insulin (μU/ml)	C peptide (pmol/L)
Good	27	47 ± 5	17 ± 3	535 ± 91
Poor	22	51 ± 7	41 ± 11	940 ± 158
Statistical difference		NS	p < 0.025	p < 0.0125

The amniotic C peptide and insulin levels are also significantly correlated with fetal morbidity and maternal metabolic control. It is likely that the new model used here (that is, the collection of amniotic fluid after arginine) may have eliminated some of the differences found by other authors.

In particular, a clear correlation between amniotic fluid insulin and C peptide concentrations has been observed in the amniotic fluid of macrosomic infants of diabetic mothers where the two peptides showed the same prognostic value.

High levels of insulin and C peptide in amniotic fluid suggest the possibility that hyperplasia or hyperactivity of fetal islet beta cells is promoting an excessive secretion of endogenous insulin. This excessive secretion may be due either to the degree of maternal diabetes according to the White classification or to poorly controlled maternal diabetes. Our findings suggest that impaired maternal metabolic control plays a prominent role during pregnancy. In this context it is worth noting that as many as 13 of 22 diabetic women from White Class A or B had poor maternal metabolic control. This observation shows how easily the subjects in these two classes (most of them were not insulin dependent when not pregnant) may be treated either inappropriately or too late. Therefore, in the management of diabetic pregnancies an optimal metabolic control throughout pregnancy is the most important factor in decreasing perinatal mortality and morbidity. Indeed the tight control of maternal blood glucose levels has already been reported to be associated with a low perinatal mortality rate in diabetic pregnancy.^{15, 16} However, it is also important to underline the fact that neonatal complications, such as hypocalcemia and hypoglycemia. associated with higher amniotic fluid insulin and C peptide levels can be related to a beta cell hyperfunction even in normal weight infants of diabetic mothers.

Finally it should be noted that even the lowest values of amniotic fluid insulin and C peptide of those infants of diabetic mothers with no neonatal complications were about twice those observed in the amniotic fluid of normal infants. This suggests that during diabetic pregnancy the fetal beta cell hyperfunction is possibly due to other causes in addition to the poor maternal metabolic control.

In conclusion the measurements of amniotic fluid insulin and C peptide after arginine stimulation have proved to be useful for the prediction of fetal outcome in diabetic pregnancy. Further studies are needed before broad clinical implications can be accurately defined.* Nevertheless the assessment of insulin and C peptide in the amniotic fluid during pregnancy can reveal valuable physiopathologic aspects and provide a useful prenatal diagnostic technique for monitoring the impact of maternal diabetes on the fetus. Both can help devise the most effective plan of diabetic management and may lead to a further reduction in perinatal mortality and morbidity in diabetic pregnancies.

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Decreased levels of amniotic fluid α -fetoprotein associated with Down syndrome

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Low maternal serum α -fetoprotein levels have been associated with fetal aneuploidies. Amniotic fluid α -fetoprotein levels have been reported to be low with Down syndrome (trisomy 21) but not with other fetal trisomies. We compared the amniotic fluid α -fetoprotein levels from 25 cases of autosomal trisomy (18 of trisomy 21, four of trisomy 13, three of trisomy 18) diagnosed by midtrimester fetal cytogenetic studies with those from matched, cytogenetically normal pregnancies. With these normal pregnancies used as controls, statistical analyses were performed on the data for all the trisomic fetuses, on the data for trisomy 21 only, and on the data for trisomies 13 and 18 combined. Amniotic fluid α -fetoprotein levels were significantly lower in the 25 trisomic cases compared with controls, 0.77 \pm 0.34 versus 1.03 \pm 0.34 mg/dl (p < 0.001). However, further analysis revealed that the difference was due to the trisomy 21 data alone. In the trisomy 21 cases there was a significant difference for α -fetoprotein levels between cases and controls (p < 0.001), whereas there was no difference for the combined trisomy 13 and 18 cases compared to controls (p > 0.40). These findings suggest that the low maternal serum levels of α -fetoprotein reported in cases of Down syndrome may be related to reduced amniotic fluid concentrations. However, the reduced maternal serum α -fetoprotein levels reportedly associated with trisomies 13 and 18 do not seem to be explained by low amniotic fluid concentrations. (AM J OBSTET GYNECOL 1985;153:541-4.)

Key words: Amniotic fluid α -fetoprotein, maternal serum α -fetoprotein, fetal aneuploidies

Increased levels of a-fetoprotein measured in maternal serum and amniotic fluid during the midtrimester of pregnancy are associated with fetal anomalies, especially open neural tube defects (anencephaly and spina bifida). Prenatal screening programs to measure midtrimester maternal serum α-fetoprotein levels have been established to identify those pregnancies at risk for neural tube defects. Diagnostic amniocentesis with measurement of amniotic fluid α-fetoprotein is recommended for those screened pregnancies found to have elevated maternal serum levels. An association has been reported between low levels of maternal serum α fetoprotein and chromosomal aneuploidies.2.3 More recently, Down syndrome (trisomy 21) has been associated with decreased a-fetoprotein levels in both maternal serum and amniotic fluid.3.4 Decreased amniotic fluid α-fetoprotein concentrations in trisomic pregnancies could explain the reported association between low levels of maternal serum α -fetoprotein and fetal trisomies. However, other studies have failed to establish a correlation between amniotic fluid and maternal serum α -fetoprotein levels, either in normal gestations or in those pregnancies with fetal chromosomal aneuploidy. We reviewed our cases of autosomal trisomies diagnosed by midtrimester fetal cytogenetic studies to determine whether amniotic fluid α -fetoprotein levels were decreased in these pregnancies when compared to those in a matched group of pregnancies with normal fetal cytogenetic studies.

Material and methods

The records of all autosomal trisomies that were diagnosed prenatally in the Laboratory of Medical Genetics at The University of Alabama at Birmingham from January, 1979, to September, 1984, were retrospectively reviewed. The prenatal diagnosis program at The University of Alabama at Birmingham is a cooperative effort between the Department of Obstetrics and Gynecology, which provides diagnostic ultrasound and amniocentesis services, and the Laboratory of Medical Genetics, which provides genetic counseling and laboratory services. Most of the midtrimester amniotic fluid samples that are cultured and karyotyped by this laboratory are obtained at our institution from patients

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Table I. Results in 25 pregnancies with an auro omal trisomy and 75 controls

Variable	Grou <u>t</u>	n	Mean	SD	<i>p</i> *
Maternal age (yr)	Trison	25	38.4	3.7	NS
	Contro	75	37.4	2.5	
Parity	Trisom=	25	2.7	2.9	NS
	Control	75	1.8	1.2	
Gestational age (days)	Trisom ·	25	119.3	7.4	NS
	Control	75	116.8	5.9	
Biparietal diameter (mm)	Trisom-	25	36	0.42	NS
	Control	66	36	0.33	

^{*}Probability, based on t test.

referred for consultation. Each patient who undergoes an amniocentesis for fetal karyotype is offered are ricitic fluid α-fetoprotein determination. Prior to each amniocentesis, an ultrasound examination was performed to document fetal viability, to confirm gestational age, to detect multiple gestation, and to choose the armiocentesis site. Transplacental puncture was avoiced whenever possible. The amniocentesis was perferred with a 22-gauge spinal needle. The initial 2 or 3 ml of amniotic fluid was discarded. From the 30 ml of amniotic fluid that was then removed, 2 ml was sert for α-fetoprotein determination. The remaining amaintic fluid was centrifuged and the cells initiated into culture. Cytogenetic studies were performed according to standard techniques. All α-fetoprotein samples were maled to the same reference laboratory (AM). All α-fetcp==tein determinations were done by rocket electronmunodiffusion, for which the normal ranges at each second-trimester week have been published.6

This study was restricted to singleton pregnances and only to those women who had amniocentesis Derformed at our institution. Each case of trisomy was matched with three controls, each of which had yie Head a normal fetal karyotype from nonbloody amn ⇒ £ fluid. The trisomies and controls were matched for race, fetal sex, gestational age (within I week), mate nel age (within 5 years). Each control chosen was from a woman who had amniocentesis in close temporal proximity to the amniocentesis that had disclosed the 1risomic fetus. Adequate follow-up data were available fcr all controls. Other variables that were tabulated included parity, biparietal diameter determined by ularasound, placental localization, the number of needle irsertions at the time of amniocentesis, and the physican who performed the amniocentesis. Gestational age at time of delivery and birth weight were examined in the control group. Gestational age was defined by last menstrual period unless there was a 10-day or greater ascrepancy in the estimated gestational age by fetal i parietal diameter obtained during the ultrasound examination. In cases of such a discrepancy the gestational age defined by ultrasound was used. Amniocenteses were performed between 15½ and 18 gestational weeks, so defined.

Results are reported as the means and standard deviation of the means. Univariate tests were made on the data with either contingency tables or t tests used as appropriate for categorical or measured responses. These tests were done in order to ascertain that the two groups were comparable for variables other than the one of primary interest (α -fetoprotein). An analysis of variance was run to test the equality of the mean αfetoprotein response for the two groups after the effects of matching were blocked out (repeated measures analysis of variance). The correlation of α -fetoprotein with the ultrasound parameters was assessed with Pearson correlation coefficients. The above analyses were run for all the data collected, on the data for trisomy 21 only, and on the combined data for trisomies 13 and 18. A probability level < 0.05 was considered significant.

Results

A total of 36 cases of autosomal trisomy were diagnosed during the study period. Ten cases were excluded, either because the samples of fluid were sent in from outside offices (three trisomy 21, two trisomy 18), because no α -fetoprotein determination was done (one trisomy 18, one trisomy 13), or because the fetus had an omphalocele (two trisomy 18, one trisomy 13), which is known to be associated with an elevated amniotic fluid α-fetoprotein concentration.⁷ Another case of trisomy 18 was excluded because of advanced gestational age, for which no appropriate controls were available. Therefore, 25 pregnancies with an autosomal trisomy (18 trisomy 21, four trisomy 13, three trisomy 18) were compared with 75 controls. All the Down syndrome cases were trisomy 21 except one, which was a translocation. For each of the 100 patients, only one amniotic fluid sample was needed for successful karyotyping and α-fetoprotein determination.

Analysis of the outcome of the 75 control pregnancies revealed a mean gestational age of 278 ± 11 days at

			α-Fetoprotein (mg	ddl		
Group	n	Mean :	SD	Range	<i>p</i> *	
Trisomy	25	0.77	0.34	0.29-1.90	< 0.001	
Control	75	1.03	0.34	0.52-2.44	10.001	
Trisomy 21	18	0.70	0.24	0.29-1.09	< 0.001	
Control	54	1.02	0.27	0.52-1.56	~0.001	
Trisomies 13 and 18	7	0.95	0.52	0.41-1.90	>0.40	
Control	21	1.08	0.49	0.55-2.44	, 0.10	

^{*}Probability, based on analysis of variance.

delivery. There were two preterm births, both occurring between 36 and 37 weeks. The average birth weight was 3445 ± 467 gm. Two babies weighed <2500 gm (2041 and 2098 gm). Six babies weighed >4000 gm, the largest weighing 4554 gm. On the basis of written follow-up reports from the referring physicians, none of these 75 babies was reported to have a congenital anomaly.

There were no differences between the trisomy and control groups with regard to maternal age, parity, gestational age at time of amniocentesis, or ultrasound biparietal diameter (Table I). Parity tended to be higher in the trisomy group but this did not reach statistical significance (p = 0.15). Of the amniocenteses, 84% from the control group and 90% from the trisomy group were done by one of the authors (R. O. D.). Three of the trisomies occurred in black patients, and the remainder in white ones. Eleven fetuses in the trisomy group were female, and 14 were male. Thirtythree control fetuses were female and 42 were male. Amniotic fluid α-fetoprotein levels were negatively correlated with biparietal diameter (r = 0.55, p < 0.001). Placental location (anterior versus posterior or fundal) had no effect on α -fetoprotein levels (p > 0.40). There was no difference between groups in the number of amniocentesis attempts necessary; only one needle insertion was required for 91% of the trisomy group and for 96% of the control group (p = 0.39).

Amniotic fluid α -fetoprotein levels were significantly lower in the trisomy group compared with controls, 0.77 ± 0.34 versus 1.03 ± 0.34 mg/dl (p < 0.001). Moreover, further analysis revealed that this difference was due to the trisomy 21 data. In the cases of trisomy 21, there was a significant difference for α -fetoprotein levels between the groups (p < 0.001), whereas there was no difference for the combined trisomy 13 and 18 data (p > 0.40) (Table II).

Comment

We found lower amniotic fluid α -fetoprotein levels in the group of Down syndrome fetuses than in

matched controls but not in the combined group of fetuses with trisomy 13 or 18. The finding that amniotic fluid α -fetoprotein was negatively correlated with fetal biparietal diameter was anticipated, since amniotic fluid α -fetoprotein levels decrease with advancing gestational age. Other than the abnormal karyotype, there was no identified explanation for the decreased amniotic fluid levels found in association with a trisomy 21 fetus. These studies suggest that α -fetoprotein production may be decreased in the fetus with Down syndrome.

The physiologic characteristics of α -fetoprotein have been extensively described.^{1,8} Briefly, α-fetoprotein is synthesized initially by the yolk sac, then by the fetal gastrointestinal tract and liver. In the second trimester, the predominant site of production is the fetal liver. The fetal plasma level peaks between 10 and 13 weeks and then progressively declines until term. α-Fetoprote.n is excreted in fetal urine and thus into amniotic fluid. Peak concentrations of amniotic fluid a-fetoprote.n occur at 12 to 14 weeks' gestation. Conversely, the maternal serum α-fetoprotein levels rise steadily until 28 to 32 weeks of gestation. Since fetal serum and amniotic fluid α-fetoprotein levels are falling while maternal serum levels are rising, a simple diffusion mechanism from one compartment to another is unlikely. In fact, Barford et al.5 found no correlation between maternal serum and amniotic fluid α-fetoprotein at 16 tc 17 weeks' gestation in normal pregnancies. However, ir conditions of markedly elevated amniotic fluid αfetoprotein content, such as an encephaly or open spina bifida, maternal serum levels are markedly elevated.1 Screening programs for neural tube defects based on measurement of maternal serum a-fetoprotein levels are already under way in many areas and are currently being advocated and developed in others.1, 9-11

Recent articles have focused attention on the significance of markedly decreased serum α -fetoprotein levels in association with fetal chromosomal abnormalities. ^{2, 5} Merkatz et al. ² reported an association between decreased maternal serum α -fetoprotein levels and fe-

tal aneuploidies. They found all 12 maternal scram αfetoprotein samples associated with trisomy 2 (two cases) and trisomy 18 (10 cases) were below the sstablished median for maternal serum α-fetoproter. Maternal serum α-fetoprotein levels were below the median in 16 of 20 cases of trisomy 21. They foated no association between amniotic fluid α-fetoprotex and any fetal chromosomal aneuploidy. However, Cackle et al.3.4 found lower levels of both amniotic fluid and maternal serum α-fetoprotein to be associated with Down syndrome. Subsequently, Nelson and Peersen¹² reported low levels of amniotic fluid α-fetopreteir in their Down syndrome cases but not in other clramosomal aneuploidies. Our study thus supports the findings of Nelson and Petersen and suggests that the reported decreases in maternal serum α-fetopromir associated with Down syndrome could be related to decreased fetal production of α -fetoprotein.

The mechanisms by which maternal serum a-fetoprotein levels are reduced in a pregnancy witl 1 trisomic fetus are not clear. Possible causes of low resternal serum α-fetoprotein in a trisomic pregnancy m ght include decreased production, increased degradation, alterations in molecular structure of α-fetoprotizing or decreased permeability of the trisomic placena and membranes. Decreased fetal production in Down syndrome is suggested by our study and other previous studies. However, to our knowledge, fetal seran afetoprotein in Down syndrome has not been remared. A mechanism other than decreased fetal production of α-fetoprotein is likely to be operative in trisonies 13 and 18, as amniotic fluid levels seem to be narral. Using isoelectric focusing, ion exchange chromatography, and agarose electrophoresis. Alpert et al. " lasive described two molecular forms of a-fetoprotein. Aterations of molecular structure could lead to an a tered rate of degradation or permeability across membranes. Alternatively, since the placenta and membran:s are genetically abnormal, there may be changes in ± €mbrane permeability, so that fetal a-fetoprotein might not have normal access to the maternal compartment.

Although the mechanism remains controversial, decreased levels of maternal serum α-fetoprotein are clearly associated with fetal chromosomal abnormalities. On the basis of low levels of maternal erum α-fetoprotein, Baumgarten et al. 4 found 4.9% of screened pregnancies in women less than age 35 to be at risk for chromosomal aneuploidies. The ratio for chromosomal aneuploidy in the group at risk was an 63. The use of low maternal serum α-fetoprotein in women under age 35 has been estimated to identify

20% to 25% of Down syndrome pregnancies in this group.³ Therefore, obstetricians must be prepared to counsel, concerning an increased risk of chromosomal aneuploidy, their patients with a low serum α -fetoprotein level.

Although from a statistical point of view we failed to show low amniotic fluid α -fetoprotein levels to be associated with trisomies 13 and 18, our numbers were small. Interestingly, five of seven α -fetoprotein values in this group were below the mean for the controls. Nelson and Petersen, 12 studying 11 such cases without anterior abdominal wall defects, found the α -fetoprotein levels to be evenly distributed about the mean of the controls. Further studies correlating maternal serum, amniotic fluid, and fetal serum α -fetoprotein content and molecular structure should aid in the elucidation of the relationship between autosomal trisomy and α -fetoprotein.

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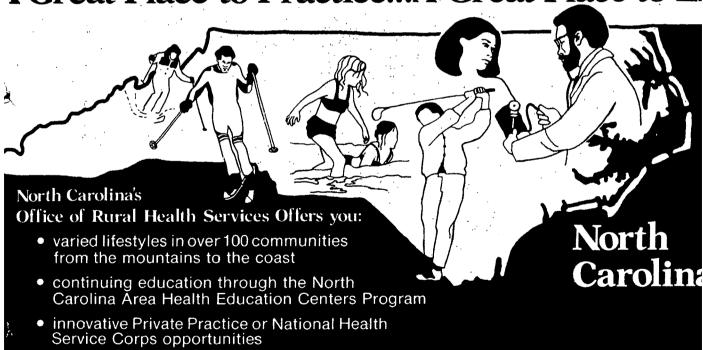
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Dickey RP: Managing Contraceptive Pill Patients, 3rd ed., Durant, Oklahoma, Creative Infomatics, Inc., 1983.
 Rates are derived from separate studies from different investigators in several population groups and cannot be compared precisely.
 Serious as well as minor adverse reactions have been reported following the use of all oral seateness the serious as well as minor adverse reactions have been reported following the use of all oral.

confraceptives.

‡Data on file for 22,489 total cycles, Wyelh Laboratories. See Important Information on following page.

IN ARIFF.

Indications and usage—LO/OVRAL* is indicated for the prevention of pregnancy in women and elect to use oral contraceptives (OC's) as a method of contraception.

Contraindications—OC's should not be used in women with any of the following conditions:

- traindications—OC's should not be used in women with any of the following conditions:

 Thrombophlebitis or thromboembolic disorders.

 A past history of deep vein thrombophlebitis or thromboembolic disorders.

 Cerebral-vascular or coronary-artery disease.

 Known or suspected carcinoma of the breast.

 Known or suspected carcinoma of the breast.

 Known or suspected bregen-dependent neoplasia.

 Undiagnosed abnormal genital bleeding.

 Known or suspected pregnancy (see Warning No. 5).

 Benign or malignant liver tumor which developed during use of OC's or other estrogen containing products.

Warnings

Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) an 1 = quite marked in women over 35 years of age. Women who use oral contraceptives should be strongfinely invised not to smake

not to struct.

The use of oral contraceptives is associated with increased risk of several serious conditions, —cuding thromboembolism, stroke, myocardial infarction, hepatic adenomal galibladder disease, hyperelasion. Practitioners prescribing oral contraceptives should be familiar with the following information r. L. Ling to these sizes.

1. Thromboembolic Disorders and Other Vascular Problems—An increased risk of thromboembolic disease associated with use of OC's is well established. Three principal studies in Greal Britain and 3 in the U.S. have demonstrated increased risk of fatal and nor fatal venous thromboembolis n and stroke, both hemorrhagic and thrombotic. These studies estimate that users of OC's are 4 to 11 time more likely than nonusers to develop these diseases without evident cause.

CEREBROVASCULAR DISORDERS—In a collaborative Arreiran study of cereprovascular disinglers in women with and without predisposing causes, it was estimated that the risk of hemorrhage stroke 4.5.2.0 times greater in users than nonusers and the risk of thrombotic stroke was 4 to 9.5 times greater in users.

many activities greater in the first of understoods associated with use of OC's has been extended with the greater for underlying risk factors for coronary artery disease (organette extended has hypertension, hypercholesteroter ia, obesity, diabetes, history of pre-ec amplic toxemia) the highest of its of developing MI, regardless of whether the patient was an OC user or not. OC's, however, were four to be a clear additional risk factor. In terms of relative risk, it has been estimated that OC users who do not one can be considered a major predisposing condition to MI) are about twice as likely to have a fabit MI as nonusers who do not smoke. OC users who are also smokers have about a 5-fold increased risk of "exit in compared to users who do not smoke, but about a 10- to 12-fold increased risk cor pared to not user. Who do not smoke. Furthermore, amount of smoking is also an important factor. In determining imports, ce of othese relative risks, however, baseline rates for various age groups must be given serious consideration importance of other predisposing conditions mentioned above in determining relative and absoluter sixs has not as yet been quantified quite likely the same synergistic action exists, but perhaps to a lesserable many analysis of data derived from several national acverse-reaction reporting spaces. Importance of other predisposing conditions mentioned above in determining relative and absolute risk as not as yet been quantified; quite likely the same synergistic action exists, but perhaps to a lesser≘lent. RISK OF DOSE—In an analysis of data derived from several national acverse-reaction reporting spalent. RISK OF DOSE—In an analysis of data derived from several national acverse-reaction reporting spalents. British investigators concluded that risk of thromboembolism, including coronary thrombosis, is it estigated to dose of estrogen in OCs. Preparations containing 100 mcg or more of estrogen were assected—with higher risk of thromboembolism than those containing 50-80 mcg. Their analysis did suggest, Inc. ever, that quantity of estrogen may not be the sole factor involved. This finding has been confirmed in the L.⊥. ESTIMATE OF EXCESS MORTALIT™ FROM CIRCULATORY DISEASES—A large prospective study carried out in the UK estimated the mortality rate per 100,000 women per year from diseases of the circulatory system for users and nonusers of OCs according to age, smoking habits, and duration of use. Cva all excess death rate annually from circulatory diseases for OC users was estimated to be 20 per 100,700 (ages 15-34-5100,000), ages 435-49-100,1000, ages 435-49-100,1000, ages 35-49-100,1000, ages 35-49-100,

thromboembolic complications has been reported in UC users. If feasible, OC's should be discontinued at least 4 weeks before surgery of a type associated with increa ecrisk of thromboembolism or prolonged immobilization.

PERSISTENCE OF RISK OF VASCULAR DISORDERS—Findings from one study in Britain involving certovascular disease and another in the U.S. concerning MI suggest an increased risk of these conditins in users of OC's persists after discontinuation of the OC's. In the British study, risk of cerebrovascular disease remained elevated in former CC users for at least 6 years after discontinuation, in the J.S. studincreased risk of MI persisted for at least 9 years in women 40 to 49 years old who had used OC's for≡or more years. Findings in both studies require confirmation since they are inconsistent with other putilished information.

2. Coular Lesions—There have been reports of neuro-ocular lesions such as optic neuritis or retires thrombosis associated with use of OC's. Discontinue OC's if there is unexplained, sudden or gradual, parts or complete loss of vision, onset of proptosis or diplopia; papilledema; or retinal-vascular lesions, acconstitute appropriate diagnostic and therapeutic measures.

or complete loss of vision, sheet of propiosis or diplopal; papilicema: or reinal-vascular lesions, asc.

3. Carcinoma—Long-term continuous administration of either natural or synthetic estrogen in certaranimal species increases frequency of carcinoma of the breast, cervix, vagina, and liver. Certain synthetic
progestogens, none currently contained in OC's, have been noted to increase incidence of mamma y
nodules, beengn and malignant, in dogs. In humans, 3 case-control studies have reported an increased
risk of endometrial carcinoma associated with prolonged use of exogenous estrogen in postmenopaus. I
women. One publication reported on the first 21 cases submitted by physicians to a registry of cases of
adenocarcinoma of the endometrium in women under 40 on OC's. Of cases found in women without
predisposing risk factors (e.g., irregular bleeding at the time OC's were first given, polycystic ovaries), nearall occurred in women who had used a sequential OC. These are no longer marketed. No evidence ha
been reported suggesting increased risk of endometrial cancer in users of conventiona. combination or
progestogen-only OC's. Several studies have found no increased in breast cancer in women withing OC's o
estrogens. One study however, while also noting no overall increased risk of breast cancer in women taking OC's o
estrogens. One study however, while also noting no overall increased risk of breast cancer in women taking OC's or
occurrence of beingn breast tumors in users of OC's has been well documented. In summary, there is a present no confirmed evidence from human studies of increased risk of cancer associated with OC's. Close
clinical surveillance of all woman on OC's is, nevertheless, essential. In all cases of undiagnosed persisten
or recurrent abnormal agina bleeding, appropriate diagnostic measures stroubles, fibrocystic disease,
or abnormal mammograms should be monitored with particular care if they elect to use OC's.

4. Hepatic Tumors—Benign hepatic adenomas have been found to be associated with

or abnormal mammograms should be monitored with particular care if they elect to use OCs.

4. Hepatic Tumors—Benigh hepatic adenomas have been found to be associated with use of OC's. One study showed that OC's with high hormor all potency were associated with high risk that lower potency OC's. Although benigh, hepatic adenomas may rupture and may cause death through intra-abcominal hemorrhage. This has been reported in short-term as well as long-term users. Two studies relater isk with duration of use of OC's, the risk being much greater after 4 or more years' use. While hepatic adenoma is are, it should be considered in women presenting abdominal pala and tenderness, abdominal mass or shock. A few cases of hepatic-altitude carcinoma have been reported in womer on OC's. Relationship of these drugs to this type of malignancy is not known.

5. Use in or Immediately Precading Pregnancy, Birth Defects in Offspring, and Malignancy in Female Offspring—Use of female sex hormones—both estrogenic and progestational agents—during early pregnancy may seriously damage the offspring. It has been shown that temales exposed in utero to dierlystification, a nonsteroidal estrogen, have increased risk of developing in later life a form of vagnal or cervical cancer ordinarily extremely rare. This risk has been estimated to be of the order of 1 in 1,000 esposures or less. Although there is no evidence now that OC's further enhance risk of developing this type of malignancy such patients should be monitored with a citicular care if they elect to use OC's. Furthermore of 1948 of 1948 Moeth Loborritories.

malignancy. Male children ⊆ exposed may develop abnormalities of the urogenital tract. Although similar data are not available with use of other estrogens, it camet be presumed they would not induce similar changes. An increased risk of congenital anomalies, including heart defects and limb defects, has been reported with use of sex hormones, including OC's, in pregrancy One case control study estimated a 4.7 fold increase in risk of limb-raduction defects in Indiants exposed in utero to sex hormones (OC's, hormonal withdrawal tests for pregnancy, or attempted treatment for threatened abortion). Some exposures involved only a few days. Data suggest that risk of limb-reduction defects in exposed fetuses is somewhat less han 1 in 1,000 live births. In the rast temale sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for hese indications, and there is no e-adence from well-controlled studies that progestogens are effective. There is some evidence that triploidy and possibly other types of polyploidy are increased among abortuses from women who become pregnant scon after ceasing OC's. Embryos with these anomalies are virtually always aborted spontaneously. Whetaer there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping OC's is transpared that, for any patient who as missed 2 consecutive periods, pregnancy should be ruled out before continuing OC's. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at time of first missed period, and further use of OC's should be withined until pregnancy has been ruled out. If pregnancy is confirmed, the patient should be apprise— of the potential risks to the letus, and advisability of continuation of the pregnancy should be discussed, it is also recommended that wor en who discontinue OC's with intent of becoming pregnant use an alternate form of contraception for a per

base this. The administration of progestogen-estrogen combinations to induce withdrawal bleeding should not be used as a test of pregnar cy.

6. Gallbladder Disease—Studies report increased risk of surgically confirmed gallbladder disease in users of OCs and estrogens. In one study, increased risk appeared after 2 years' use and doubled after 4 or 5 years' use. In one of the other sudies, increased risk was apparent between 6 and 12 months' use.

7. Carbohydrate and Lipid Metabolic Effects—Decrease in glucose tolerance has been observed in a significant percentage of patients on OC's. For this reason, prediabetic and diabetic patients should be carefully observed while on OCs. Increase in triglycerides and total phospholipids has been observed in a patients on OC's, clinical significance of this finding remains to be defined.

8. Elevated Blood Pressure—Increase in blood pressure has been reported in patients on OC's. In some women, hypertension may occur within a few menths of beginning CC's. In the 1st year of use, prevalence of women with hypertension is 1bw in users and may be no higher than that of a companie group of ronusers. Prevalence in users in creases, however, with longer exposure, and in the 5th year of use is 2½ to 3 times the reported prevaler ce in the 1st year. Age is also strongly correlated with development of hypertension in OC users. Women who previously have had hypertension during pregnancy may be more likely to develop elevation of blood-pressure on OC's. Hypertension of uning pregnancy may be more likely to develop elevation of blood-pressure on OC's. Hypertension of uning pregnancy may be more likely to develop elevation of blood-pressure on OC's. Hypertension of headache of a new pattern which is recurrent, persistent, or severe, requires discontinuation of OC's and evaluation of the cause.

10. Bleeding Irregularities—Breakthrough bleeding, spotting, and a menorrhea are frequent reasons for patients discontinuing OC's. In the mind.

11. In undiagnosed persistent or recurrent abnorma

runctional causes should be borne in mind.

In undiagnosed persistent or recurrent abnormal bleeding from the vagina, adequate diagnostic measures are indicated to rule out pregnaziny or malignancy. If pathology has been excluded, time or change to another OC may solve the problem. Changing to an OC with a higher estrogen content, while potentially useful in minimizing menstrual intergularity, should be done only if necessary, since this may increase risk of thromboembolic disease. Women with past history of oligomenorrhee or secondary amenornhea or young women without regular cycles may have a tendency to remain anovulatory or to become amenornheic affection discontinuing OC's. Women with these pre-existing problems should be advised of hits possibility and encouraged to use other methods. Post-use anovulation, possibly prelonged, may also occur in women without previous irregularities.

without previous irregularities.

11. Ectopic Pregnancy.—Ectopic as well as intrauterine pregnancy may occur in contraceptive failures.

12. Breast-feeding—OC's given in the postpartum period may interfere with lactation and decrease quantity and quality of breast milk. Furthermore, a small fraction of the hormones in OC's has been identified in the milk of mothers on OC's, effects, if any, on the breast-fed child have not been determined. If feasible, defer OC's until infant has been weaned.

Precautions—GENERAL—1. A complete medical and family history should be taken prior to initiation of OC's. Pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Pap smear and relevant laboratory tests. As a general rule OC's should not be prescribed for longer than 1 year without another physical examination. 2. Under influence of estrogen-progestagen preparations, pre-existing uterine leiomyomata may increase in size.

3. Patients with history of psychic cepression should be carefully observed and the drug discontinued if depression recurs to a serious deg ee. Patients becoming significantly depressed while on OC's should stop OC's and use an alternate metit of to try to determine whether the symptom is drug-relation.

4. OC's may cause some degree of Liic retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention, such as convulsive disorders, migraine syndrame, asthma, or pardiac or renal insufficiency.

Patients with a past history of jauridice during pregnancy have an increased risk of recurrence while on OC's. If jaundice develops, OC's should be discentinued.

6. Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution.
7. OC users may have disturbances in normal tryprophan metabolism which may result in a relative pyridoxine deficiency. Chinical significance is undetermined.

Secum folate levels may be depressed by OC's. Since the pregnant woman is predisposed to development of folate deficiency and incidence of frate deficiency increases with increasing gestation, it is possible that if a woman becomes pregnant short! after stopping OC's, she may have a greater chance of developing folate deficiency and complications at ributed to this deficiency.

If a woman becomes pregnant shorth after stopping OC's, she may have a greater chance of developing folate deficiency and complications at ributed to this deficiency.

9. The pathologist should be advised of OC therapy when relevant specimens are submitted.

10. Certain endocrine- and liver-function tests and blood components may be affected by estrogen-containing OC's: a Increased sulfotormophitable in reletation. b. Increased prothombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepine phrine-induced platelet aggregability. c. Increased thyroid-binding globulin (TBG) leading to increased circulating total-thyroid hormone, ameasured by protein-bound iodine (PBI), T4 by column, or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG, free T4 concentration is unaltered, d. Decreased pregnanediol excretion.

e. Reduced response to metyrapone test.

Information for the Patient—See Patient Package Labeling.

Drug Interactions—Reduced efficacy and increased incidence of breakthrough bleeding have been associated with concomitant use of rifamp n. A similar association has been suggested with barbiturates, phenylbulazione, phenylohi sodium, amoicillin and tetracycline.

Carcinogenesis—See Warnings on carchogenic potential of OC's.

Pregnancy—Category X. See Contraind ations, Warnings.

Nursing Mothers—See Warnings.

Adverse Reactions—An increased risk of these serious adverse reactions has been associated with use of OC's (see Warnings): homopophiebits, pulmonary embolism, coronary thrombosis, cerebral thrombosis cerebral memorrhage, hypertension, galizladder disease, benign hepato mas, congenital anomalies.

The following adverse reactions have been reported in patients on OC's and are believed to be drug-related.

optic neurals.

The following adverse reactions have beer reported in patients on OC's and are believed to be drug-related. Nausea and/or vomiting, usually the most common adverse reactions, occur in approximately 10 percent or less of patients during the first cycle. Their reactions, as a general rule, are seen much less frequently or only occasionally. Gastrointestinal symptoms (such as abdominal cramps and bloating); breakthrough bleeding, spotting, change in mensitrua flow; cysmenorrhea, amenorrhea during and after treatment, temporary infertility after discontinuance of treatherit, edema; chloasema or melasma which may persist; breast changes: tenderness, enlargement, and secretion, change in weight (increase or decrease); change in cervical erosion and cervical secretion; possible diminution in lactation when given immediately postpartum; cholestatic jaundice; migraine; increase in size of uterine felomyomata; rash (allergic); mental depression; reduced tolerance to carbohydrates vaginal candidiasis; change in corneal curvature (steepening), ntolerance to contact lenses.

The following adverse reactions have beer reported in users of OC's, and the association has been neither confirmed nor refuted; premenstrual-like syndrome, cataracts, changes in libido, chorea, changes in appeite, cystitis-like syndrome, headache, ner ousness, dizziness, hirsutism, oss of scalp hair, erythema multiforms, crythema nodosum, hemorrhagic aruption, vaginitis, porphyria.

Loute Overdose—Serious ill effects have ret been reported following acute ingestion of large doses of OC's by young children. Overdosage may cause nausea, and withdrawal bleeding may occur in females.





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Michael S. Baggish, M.D.

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Two series of patients with genital warts were compared for effectiveness of tr=atment by means of the carbon dioxide laser. In the earlier series (1977 to 1981) laser vaporization wa₃ used to remove overt warts while surrounding normal skin was spared. Examination of anal, urethral and other extragenital sites for possible involvement was done only when warts persisted or recurred. In the later series (1983 to 1985), patients were similarly treated by carbon dioxide laser techniques; however, two additional measures supplemented laser vaporization of gross condyloma acuminatum. The brush technique superficially coagulated skin and mucosal surfaces contiguous to warts. This π ethodology was hypothesized to eliminate subclinical human papillomavirus infection within normal-appearing epithelium. Compulsive examination and treatment of extragenital sites at the time of the Enitial laser surgical procedure eliminated the most likely locations for persistence or recurrence. The primary cure rate for the earlier series was 65.8% compared to 91% for the later series. This difference was highly significant (p < 0.005). (AM J OBSTET GYNECOL 1985;153:545-50.)

Key words: Carbon dioxide laser, genital warts, condyloma acuminatum, papillomavirus

Human papillomavirus infections of the female genital tract produce disagreeable lesions and discomfort in pregnant and nonpregnant women. Condyloma acuminatum is among the most frequent disorders treated with the carbon dioxide laser and occurs with epidemic frequency in the United States. Genital warts appear to grow rapidly during states of relative or actual immune deficiency and similarly seem to persist or recur during such states. Pregnancy not only presents a milieu for rampant growth of warts but also accentuates the risk of vocal cord papilloma in the newborn infant. The latter were reported in 40% of children who were born to mothers having condyloma during pregnancy or at parturition.1 This risk has never been confirmed and remains only an estimate. Since the risk to the newborn infant is unknown, confusion exists as to how drastic and how aggressive treatment programs should be in pregnant women. An equally serious problem associated with human papillomavirus infections involves the relationship of this virus to lower genital tract neoplasia.2 Specific viral types have been reported to be associated very frequently with cervical intraepithelial neoplasia. Additionally, vaginal and vulvar neoplasia frequently presents as condylomatous lesions and may also be associated with typical, benign condyloma acuminatum. Published data suggest that as many as ≥5% of women afflicted with vulvar carcinoma in situ Eave been previously treated for benign condyloma accuminatum.³

The carbon dioxide laser has proved to be one of the few effective methods of therapy for condyloma acuminatum. ⁴⁻⁶ Since warts have been found in extragenital sites in 32% of resistant cases and since the carbon dioxide aser can destroy these lesions in virtually any location it follows that this requirement of therapy is essential if these lesions are to be eliminated. ⁷ Unfortunately even the carbon dioxide laser is associated with a high primary failure rate particularly when the warts are widespread and dense.

The major difficulty in eliminating the human papillomavious relates to the hypothesis that this virus resides not only in the warts but also in the surrounding normal-appearing skin and mucous membranes. This article compares the treatment of an extensive number of women with condyloma acuminatum infections of the lower genital tract within two time periods. The carbon dioxide laser was used to treat the lesions in both series; however, different treatment techniques were used.

Material and methods

Grou⊃ 1 patients numbered 228 and we're treated betweer the years 1977 to 1981.⁴ Two hundred of these women had received other treatments prior to being referrec for laser surgical procedures. These treatments included podophyllin, cautery, cryosurgery, and excisior. The severity of condyloma acuminatum was defined according to the following criteria: (1) mild,

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Table I. Description of groups 1 and 2

	,	non	ior laser ment	Preg	rnant		•	Seve	erity						nıtal war treatmen		
						М	ild	Mod	erate	Sev	vere	4 1	no	4-12	2 mo	≥12	2 mo
Group*	N	n	%	n	%	n	9	· n	%	n	%	n	%	n	%	n	%
1 2	228 100	200 62	88 62	10 15	4 15	5 4	رة ر	134 51	59 51	89 45	39 45	139 46	61 46	82 50	36 50	7 4	3 4

Group 1: Treated at Mt. Sinai Hospital, Hartford, Connecticut. Group 2: Treated at Mt. Sinai Hospital, Hartford, Connecticut, and State University of New York Upstate Medical Cenner and Crouse Irving Memorial Hospital, Syracuse, New York.

Table II. Power settings and spot size specifications for carbon dioxide laser vaporization of genital and extragenital warts

Location	Power (W)	Spot (mm)	Power dess to		
Vulva	15-50	1.5-3.0	160-2225		
Vagina	15-20	1.5-2.0	375-88∋ +		
Cervix	20-40	1.5	888-17DC		
Urethra	5-10	1.0 - 1.5	222-100C		
Anus	5-10	1.0-1.5	222-100C		
Mouth	10-15	1.5 - 2.0	250-665		
Brush	2-8	1.0-2.0	100-200		

<10 warts and no extragenital site involvement (2) moderate, >10 but <20 warts and extragenital ste involvement; (3) severe, >20 warts, confluent warts, ≠nd extragenital site involvement (Table I).

The plan of treatment during this phase was axirization of lesions to the level of the surrounding skin surface while neighboring normal-appearing extralium was spared. All women (groups 1 and 2) were on a similar postoperative regimen that included seavater sitz baths, perineal irrigation with Betadine dilued 1: 4, blow drying of the perineum, stool softeners, and vaginal douches.8 Patients were treated under local or general anesthesia depending on the extent of the lesions and the desire or lack of desire to elimina e the lesions at a single treatment session. All patients required some anesthesia for vaporization of vulvar-aral, oral, vaginal, and skin warts. The carbon dioxidelaser specifications included spots of 1.5 to 3.0 mm in diameter with power densities ranging between 160 and 2222 W/cm2 (Table II).

Group 2 patients were accrued between the years 1983 and 1985. One hundred were treated an I fellowed up during this time interval. Sixty-two women received some type of previous nonlaser treatment prior to referral; 38 women were treated only with the carbon dioxide laser.

A total of 90% of the patients were treated urger general anesthesia during a single treatment se s on. Under every circumstance, a laser speculum was paid into the rectum and the anus was scanned under ris-

croscopic guidance to determine whether anal warts were present (Fig. 1). Additionally, in every case the urethra, vagina, cervix, extremities, and mouth were examined for the presence of warts. Male partners were also examined with the colposcope used for magnification. When warts were found in genital or extragenital sites or in male partners, they were treated with the carbon dioxide laser.

When warts were extensive the carbon dioxide laser free-hand piece with a spot size of 2 to 3 mm and power settings of 30 to 50 W were used in order to rapidly vaporize vulvar and perianal warts. The laser coupled to a Zeiss opmi 1-h microscope was used not only to provide magnification but also to provide good illumination to locate and eliminate warts of the vagina, anus, urethra, and mouth. When the free-hand laser was used, $2\frac{1}{2} \times$ magnification was provided by eyeglassmounted microscopes (Designs for Vision) with a coaxial head light. The laser micromanipulator provided variable spot size ranging from 0.5 to 2 mm with powers ranging from 2 to 30 W (Table II).

The most notable difference in treatment techniques between groups 1 and 2 was the addition of the brush technique for group 2 patients.

The brush technique. The brush technique is preferred for epithelial surfaces outwardly appearing normal but contiguous to or surrounding areas of overt wart involvement. Additionally, this technique is used on skin or mucous membrane surfaces showing condylomatous change.

The brush methodology aims to blanch skin white with the laser. This technique destroys only the epidermis and spares the underlying dermis. Blistering indicates too much power and too much destruction (Fig. 2, A and B).

The technique is best accomplished with spots measuring 1.5 to 2 mm in diameter and power settings of 2 to 5 W. The estimated power density for brushing ranges between 100 and 200 W/cm². The defocused laser hand piece or the micromanipulator control stick is moved rapidly over the epithelial surface. Histologic section reveals epidermal destruction only; hence there



Fig. 1. A laser speculum has been inserted into the anus. An extensive number of warts can be seen on the posterior anal wall. Magnification and good light are essential to identify and treat these small lesions.

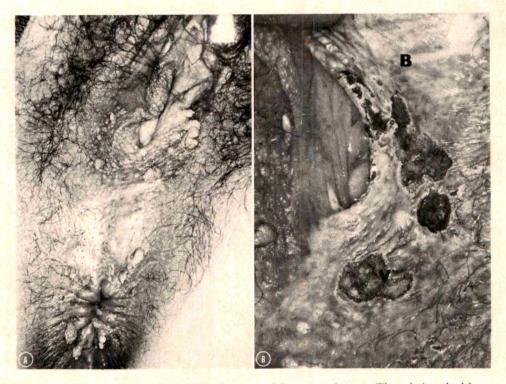


Fig. 2. A, A 26-year-old woman with moderate condyloma acuminatum. These lesions had been treated for more than I year with podophyllin, trichloroacetic acid, and cryosurgery prior to referral for laser surgery. B, Laser vaporization has been carried out to reduce the warts to skin surface level (v). "Brushing" with a low power density laser beam (B) produces coagulation of the epidermis for a 2 cm radius around the gross condyloma acuminatum.

is selective human papillomavirus destruction since the virus proliferates in the prickle or basal cell layers. Large tracts of skin may be brushed rapidly. Interestingly, patients undergoing laser surgery under local anesthesia are able to tolerate brushing even in lightly anesthetized areas. The sensation described by patients

is that of a prickly, warm feeling. The key to limiting discomfort appears to be the rapidity of laser beam movement over the skin surface.

Brushing is performed over a 2 cm radius peripheral to gress warts. Pebble-like changes of the labia minora or vagina, that is, condylomatous changes, necessitate



Fig. 3. Vaporization of anal warts is performed by aiming the laser beam between the blades of the speculum. A moistened cotton-tipped application is seen to the right and above the vaporized zone.

brushing the entire medial aspect of the labia and/or the entire vaginal mucous membrane.

Patients were followed up according to a specific schedule. The initial posttreatment examination occurred at 4 to 6 weeks. Subsequent follow-up examinations were scheduled weekly until all warts were eradicated; these visits took place in the office setting where laser treatment with the patient under local anesthesia could be carried out. Subsequent examinations were performed every 6 months for 2 years. Persistence was diagnosed when warts reappeared after treatment. In both series <7% of the women developed new warts after 6 months; therefore, persistence of disease was substantially more frequent than recurrence.

Vaporization of vulvar warts. Whether vaporization is performed with the laser free-hand piece or the micromanipulator, condylomas are vaporized from outside inward. This technique collapses the wart toward the direction of the laser beam. Vaporization is carried out to a shallow depth, thus reducing the wart to the level of the surrounding skin. If large vessels are encountered and bleeding results, the laser beam is defocused to provide better coagulation.

Vaporization of urethral warts. Fine 10-inch tita-

nium skin hooks apply traction on either side of the external urethral meatus at the 9 and 3 o'clock positions. Magnification is provided by a colposcope or microscope and the urethra is scanned for the presence of warts. With the use of low power densities the warts are carefully vaporized. Excess char is dabbed away with cotton-tipped applicators soaked in 4% acetic acid.

Vaporization of anal warts. Anal and perianal warts are vaporized with the laser beam directed via the micromanipulator. Superficial vaporization is carried out to avoid bleeding within this highly vascular area. The laser beam is aimed between the blades of a specially designed laser speculum. The speculum is progressively rotated clockwise to cover the entire expanse of the anus. Perianal warts are vaporized to the skin surface. Next, linear vaporizations are performed starting at the anal mucosal margin and extending outward with power densities of 300 W/cm². The latter technique causes the mucosa to evert and eliminates the possibility of stricture (Fig. 3).

Laser vaporization of the vagina. Warts in the lower vagina and on the anterior and posterior walls can be directly vaporized with the laser micromanipulator. The cervix should be manipulated by means of a long-handled skin hook in order to expose the vaginal fornices. A gold-coated mirror is used to reflect the laser beam so as to indirectly strike condyloma acuminatum located on the vaginal sidewalls and on the posterior aspects of the hymenal ring. Occasionally extensive micro-warty changes are seen virtually covering the entire vaginal mucosa; the brush technique is indicated in these circumstances.

Results

The most notable difference between the two study groups was the 25% improvement in the single treatment results (Tables IIIA and IIIB). Since both groups 1 and 2 were composed of similar patient populations with equivalent distribution and severity of condyloma acuminatum (Table I), the 91% primary cure rates may be explained by the initiation of the brush technique and the more frequent use of general anesthesia. The latter allowed eradication of warts to be finished during one treatment session and also facilitated examination and treatment of anal, urethral, and vaginal lesions.

In both series, male partners were investigated for the presence of warts. Fifty consorts (25%) in group 1 and 47 (47%) in group 2 were found to have genital or extragenital warts (Table IV). The increased frequency of male "pickups" in group 2 can be attributed to the lessons learned in the earlier study and alacrity in seeking out possibly afflicted men (Fig. 4). The results of therapy during pregnancy were no different than for those in nonpregnant patients, that is, the proportion of failures was similar to that of the general series. Interestingly, group 2 was more heavily



Fig. 4. A total of 47% of male consorts have genital warts. Frequently these lesions are so small and flat that they will be missed on cursory examination. With the laser directed via the micromanipulator, vaporization of these resistant warts is accomplished.

Table IIIA. Results of carbon dioxide laser treatment for genital warts

Eliminated with one laser treatment Group N n %		Follow-up								
			≤6 mo		6-12 mo		≥12 mo			
	Persistence (n)	n	%	n	%	n	%			
1 2	228 100	150 91	65.8* 91*	78 9	2 4	0.8	38 21	17 21	188 75	82 75

^{*}Highly significant at p < 0.005.

Table IIIB. Significance of results of carbon dioxide laser treatment for genital warts

Total	Persistence	Eliminated	Group
228	78 (60.48)	150 (167.52)	1
100	9 (26.52)	91 (73.48)	2
328	87	241	Total
	I 1	1 1	
	60.48 26.52	167.52 73.48	$\chi^2 = 17.02$
	$ \begin{array}{c cccc} & 1 & 1 \\ \hline & 60.48 & 26.52 \\ & + 0.016 + 0.038 \\ \end{array} $		$\chi^2 = 17.02$ = 289.68

^{= 289.68 (0.074).} = 21.436 (p < 0.005, highly significant).

weighted with pregnant patients. Four failures in group 2 (requiring more than a single treatment) occurred in immunologically compromised women.

Postoperative discomfort secondary to the brush technique was minimal and required no additional analgesia, even though rather extensive areas were treated. The brushed epithelium peeled away in 24 to 48 hours and was replaced with new tissue in 7 to 10 days.

When gross warts were widespread over external skin surfaces requiring extensive laser vaporization, post-

Table IV. Other site involvement in two series of genital warts treated by carbon dioxide laser

	Gro	up 1	Group 2		
Site	n	%	n	%	
Oral cavity	3	1.3	4	4	
Extremities	1	0.4	10	10	
Anus	83	36	80	80	
Urethra	12	5.3	33	33	
Male consorts	50	22	47	47	

operative discomfort was usually delayed for 4 to 7 days. The severity of postvaporization pain as compared to postbrush pain was definitely increased. Although "instant ocean" sitz baths greatly alleviated discomfort, the nature of open-to-air wounds tended to create more discomfort than covered wounds. Approximately 15% to 20% of these perineal wounds were covered with Bioclusive urethane dressings (Johnson & Johnson) resulting in substantial amelioration of pain. Unfortunately, because of excretory requirements and persistent vaginal discharge, these dressings did not easily remain in place. The wounds required frequent redressing. Nevertheless, the urethane dressings definitely promoted accelerated wound healing and diminished pain.

No patient among the total of 328 women undergoing treatment with the carbon dioxide laser developed an infection. Similarly, all wounds healed without scar formation. No anal or urethral strictures were observed or reported.

Comment

Characteristically, human papillomavirus infections especially of the lower genital tract have a tendency to stubborn persistence. Afflicted women find genital warts abhorrent and wish to have the lesions expeditiously removed. Not only are there compelling psychological reasons for eliminating condyloma accuminatum, but the additional risks of venereal transmission, neoplasia, and newborn vocal cord papillomas require the best treatment available. Although a wide variety of chemical and surgical remedies have been used by gynecologists over the years, none have proved safe and effective. Recently substantial experience has been gained with the carbon dioxide laser used to vaporize condyloma acuminatum. Although the cure rates have been reasonably good, about 90%, multiple treatments are commonly required. The data presented here provide a logical answer to the vexing question of subclinical human papillomavirus located in otherwise normal-appearing epithelium. The improved pumary cure rates substantiate the need to eliminate the subclinical virus and reduce the chance of persistence.

It is probable that the ≥100° C temperatures produced by the carbon dioxide laser beam eliminate the virus producing the infection. Previously published data also showed that this technique could eliminate the herpes simplex virus from infected tissue.⁷

Histologically the brush technique thermally coagulates the epidermis while creating minimal or no thermal damage to the underlying dermis. This treatment regimen, unique to the laser, eliminates the habitat in which the human papillomavirus multiplies; therefore, the therapeutic basis for this technique of laser intervention is grounded on a sound premise. Since wide tracts of tissue may be brushed with minimal discomfort

and with no demonstrable retardation of healing, this new methodology is very attractive. Clearly, the elimination of the wart without consideration of contiguous epithelial surfaces must now be considered obsolete.

This study has shown that genital warts create widespread infection not only affecting the vulva but also encroaching on the vagina and cervix. Extragenital sites, when examined with the aid of an operating microscope, commonly show involvement: 80% in the anus, 33% in the urethra, and a smaller percentage on remote skin and mucosal surfaces (Table IV). Therefore, every woman with genital condyloma acuminatum infections must have these "high-risk" locations closely examined and treated. Since male consorts (group 2) were infected 47% of the time, the genitalia and perianal skin of these men must be examined with the use of magnification and good light. Since the relationship of human papillomavirus to lower genital tract neoplasia is at least circumstantially probable, a sample biopsy should be obtained in every case. In particular, suspicious lesions, that is, flat and pigmented warts, should have multiple samples taken.

Finally carbon dioxide laser treatment is a skilled procedure and cannot simplistically entail merely "blasting" warts off the surface of the skin. Proper power settings and spot sizes are required for the most advantageous ablation of the lesions.

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Cytochrome P-450 activity in human leiomyoma and normal myometrium

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Variations in cytochrome P-450 levels may influence the responsiveness of uterine and breast tissue as well as carcinomas to endocrine therapy and may be of particular importance with agents such as tamoxifen (Nolvadex) where hydroxylation is known to alter therapeutic activities. Therefore, a sensitive spectrophotometric assay of cytochrome P-450 levels in reproductive tissue microsomes was developed to measure cyclohexane hydroxylase activity. Cyclohexane served as a substrate for several forms of cytochrome P-450. Human uterine leiomyomas (uterine fibroid tumor) contained significantly higher (p < 0.01) cytochrome P-450 activity than adjacent normal myometrium. Specific activities for both leiomyomas (2.87 \pm 0.26 nmol/min/mg) and normal myometrium (1.60 \pm 0.1" nmol/min/mg) were in the range of those observed for untreated rabbit liver microsomes (1 to 3 nmol/min/mg). The contribution of smooth muscle in the specimen, the phase of the menstrual cycle, and the clinical diagnosis did not influence the level of cytochrome P-450 activity. (AM J OBSTET GYNECOL 1985; 53 551-5.)

Key words: Uterus, leiomyoma, cytochrome P-450 hydroxylation, m/ometrium, microsomes, tamoxifen, liver

In 1962, Omura and Sato¹ introduced the name cytochrome P-450 to designate a carbon monoxide-binding pigment present in liver microsomes. Cytochrome P-450 serves a vital role as the terminal oxidase in the microsomal monooxygenase (hydroxylation) reactions of a wide variety of xenobiotics including drugs, pesticides, carcinogens, and environmental pollutants, as well as endogenous substrates such as steroids. The hydroxylation is carried out with molecular oxygen and reducing equivalents from reduced nicotinamide adenine dinucleotide phosphate (NADPH) that are shuttled through a flavoprotein, cytochrome P-450 reductase. In mammals, the highest concentrations of cytochrome P-450 are in liver, but these heme proteins are also found in the endoplasmic reticulum of many other cell types including brain, kidney, colon, lung, adrenal cortex, skin, testis, placenta, and more recently breast.2.3

Not only does cytochrome P-450 exist in most tissues, but distribution studies have revealed that the enzyme can be detected in almost all forms of life. However, very few investigations have been performed concerning the existence and role of cytochrome P-450 in normal and neoplastic reproductive tissues (see references 4 to 6). Furthermore, the probable involvement of cytochrome P-450 in carcinogenesis by conversion of procarcinogens into carcinogens, local metabolism of chemotherapeutic drugs, or estrogen synthesis by breast or utering tumors remains to be demonstrated in these tissues.

Our interest lies in the possibility that cytochrome P-450 may play a role in determining the responsiveness of human uterine tumors as well as breast neoplasms to additive endocrine therapy. It has been demonstrated that the presence of the estrogen receptor in breast tumors is a good predictive index of response to endocrine therapy. However, in a significant fraction of patients in whom tumors exhibit the estrogen receptor, no response to endocrine therapy is observed. Wittliff? has suggested earlier that this lack of response may be due to "defective" estrogen receptors in certain tumors.

It is well established that liver microsomal cytochrome F-450 is involved in steroid metabolism and may also participate in the metabolism of antiestrogens such as tamoxifen. Thus it is possible that the presence of high concentrations of this enzyme in breast and uterine tumors may result in metabolism of sex steroids and steroid derivatives, thereby rendering them inactive before interaction with the estrogen receptor. Determination of the levels of cytochrome P-450 in human breast and uterine carcinomas in conjunction with es-

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trogen and progesterone receptor assays may serve as an additional indicator of the efficacy of administrative endocrine therapy.

In the present study, cyclohexane was used as a substrate to assay for cytochrome P-450—dependent hydroxylation activity in normal and neoplastic (leic myoma) uterine tissue. The alicyclic hydrocarbon combexane was chosen as substrate in these studies because of its high turnover number, its high efficiency a hydroxylation, and the fact that cyclohexane acts as a general substrate for most forms of cytochrome F-450 in rabbit liver microsomes. Cytochrome P-450 ac nity (cyclohexane hydroxylase activity) is readily modified spectrophotometrically by measuring NADPH oxidation at 340 nm.

In an earlier study, in order to validate the NADPH assay as well as to confirm that NADPH oxidation is coupled to cyclohexane hydroxylation, an isotope dilution—gas chromatography/mass spectrometry pacedure was developed for detecting low levels of the hydroxylated product, cyclohexanol, in rabbit line: microsomes as well as human breast and attaine microsomes. Furthermore, we also demonstrated in human breast tumors that this NADPH oxidation is completely inhibited by carbon monoxide and or an antibody against rat liver cytochrome P-450.

In this study, we assayed microsomes from human uterine leiomyomas for cytochrome P-450—derement hydroxylation activity and compared this with the enzyme activity observed in normal uterine ribrosomes from patients with several gynecologic diomers. Smooth muscle content was also determined in most of the tissues and related to cytochrome P-450 activity. Furthermore, the two phases of the mensurual cycle were compared with respect to enzyme activity in both leiomyomas and myometrial tissues.

Material and methods

Uteri from nonpregnant, premenopausal vomen (age range 20 to 48 years), undergoing hysterectomy for medically indicated reasons, were obtainec from the operating room and immediately placed ir ice-cold physiologic saline solution. After examination = the pathologist, tissue sections were either used immediately or stored frozen in saline solution at $-9C^{\circ}C$. If a leiomyoma was present, one section of the neorlasm and one of the adjacent normal myometrian were taken from each uterine specimen. The fro en uteri were dissected free of peritoneum and endcmetrium and divided longitudinally at the midline. Eacl half was subdivided into approximately equal anterio-, lateral, and posterior strips. Each strip was sectioned no 1 cm segments, and a representative portion of each section was immediately placed in formalin for histologic study. In many instances a peritoneum- and endomeriumfree section of myometrium was taken from the uterine fundus and was used for cytochrome P-450 analysis.

Histologic features. Uterine segments were embedded in paraffin and sectioned (4 µm thickness). Smooth muscle content was determined by staining segments with trichrome, 10 according to an area sampling technique modified by the method of Weibel. 11 Segments were randomly sampled at 100 µm intervals and categorized as smooth muscle or non—smooth muscle. The smooth muscle content was expressed as a percentage of the total sample. Each segment was evaluated in triplicate, and at least 150 points were taken per evaluation. Triplicate determinations that did not fall within theoretical precision (Poisson distribution) were rejected, and the determinations were repeated.

Specimen dating and diagnosis. The phase of the menstrual cycle was determined according to the patient's last normal menstrual period and histopathologic evaluation. Diagnosis was taken from the patient's chart. Since uteri (normal and containing leiomyoma) were received as whole tissue samples, mincing was required before homogenization.

Preparation of tissue cytosols. Tissue cytosols were prepared at 0° to 3° C. Cold buffer consisting of 10 mmol/L Tris hydrochloride, pH 7.6, containing 1.5 mmol/L ethylenediaminetetraacetic acid, 10 mmol/L monothioglycerol, and 10% glycerol, was added to the minced tumor at a volume determined by the tissue wet weight (1:3, w/v). Tissues were homogenized with several intermittent 5- to 10-second pulses of the Polytron (Brinkman Instruments, Lucerne, Switzerland) homogenizer. The homogenate was centrifuged at $105,000 \times g$ for 30 minutes in fixed angle rotors in a preparative ultracentrifuge. The supernatant was drawn carefully from beneath the lipid layer with a Pasteur pipette and used in steroid receptor assays⁷ for studies such as those described previously.3 The pellet was frozen and later processed for microsome prepa-

Preparation of tumor microsomes. Pellets from the cytosol preparation described above were thawed immediately before assay. The pellets were resuspended in a volume equivalent to four times the original tissue weight of 10 mmol/L Tris hydrochloride buffer, pH 7.6, containing 2.5 mmol/L ethylenediaminetetraacetic acid and 10% glycerol (v/v). At least 0.3 gm of tissue was necessary to yield a sufficient amount of microsomes for each assay.2 The suspension was homogenized with a Polytron at a setting of 7 for two 5-second bursts. The homogenized membranes were centrifuged at $10,000 \times g$ for 20 minutes and the supernatant was then centrifuged at 230,000 × g for 30 minutes to isolate microsomal membranes. The pellets were resuspended (20 strokes in a 1 ml glass pestle tissue grinder, Kontes Glass Co., Vineland, New Jersey) in 0.6 ml of

Table I. Summary of cytochrome P-450 activity in human uterine leiomyomas and adjacent normal myometrium

Tissue	Specific astivity (nmol/min/mg)	n
Human unterine leiomyoma (all patients)	$2.87 \pm 0.26*$	17
Proliferative phase	3.03 ± 0.47	7
Secretory phase	2.76 ± 0.32	10
Normal myometrium (all patients)	1.60 ± 0.11	30
Proliferative phase	1.55 ± 0.14	18
Secretory phase	1.68 ± 0.20	12
Normal myometrium (classified according to gynecologic disorder†)		
Abnormal uterine bleeding	1.62 ± 0.21	14
Urinary stress incontinence	1.56 ± 0.43	3
Dysmenorrhea	1.94 ± 0.34	7
Uterine descensus	1.38 ± 0.28	5
Rabbit liver‡		
Untreated	1-3	
Phenobarbital-induced	7-20	

^{*}Mean ± SE. Summary values presented in a preliminary report.13

3

50 mmol/L Trishydrochloride buffer, pH 7.6, containing 1 mmol/L ethylenediaminetetraacetic acid and 20% glycerol. The protein concentration of this final suspension was 1 to 5 mg/ml as determined by the procedure of Lowry et al.12 with bovine serum albumin used as a standard. Microsomes could be stored at -20° C for more than a month without loss of cyclohexane hydroxylase activity.2 However, microsomes may be thawed only one time; considerable loss in activity was observed after repeated freeze-and-thaw cycles. Liver microsomes from phenobarbital-treated rabbits were prepared as previously described by Wagner et al.13

Cyclohexane hydroxylation assay. Hydroxylase activity by uterine microsomes was determined by measuring cyclohexane-dependent NADPH oxidation.2 The assay mixture was prepared by adding 100 µl of the microsomal suspension to 1.74 ml of solution containing a final concentration of 50 mmol/L HEPES buffer at pH 7.0, 15 mmol/L magnesium chloride, and 0.17 µmol/L NADPH (Sigma Chemical Co., St. Louis, Missouri). The mixture was divided into two 0.89 ml portions, and 10 µl of 1 mol/L cyclohexane (Matheson, Coleman and Bell, Norwood, Ohio) in methanol was added to one portion while 10 µl of methanol was added to the other. The reaction was carried out at 30° C. At the end of 30 minutes, 100 µl of 10% (w/v) sodium dodecyl sulfate (Sigma Chemical Co.) was added to each portion and the difference in absorbance at 340 nm between the two samples was determined on a Cary 219 spectrophotometer. Activity (NADPH oxidized) was

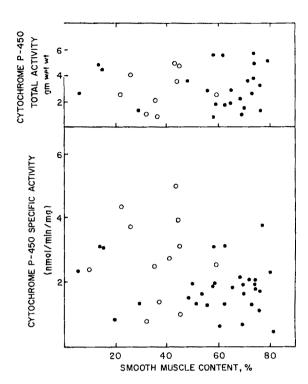


Fig. 1. Relationship between cyclohexane hydroxylation activity and smooth muscle content of human myometrium. Cyclohexana hydroxylation activity (NADPH oxidation) was compared with smooth muscle content for normal myometrium (•) and uterine leiomyomas (0). Smooth muscle content was determined as described in the Material and methods section. Each point represents the mean value of duplicate assays. Tetal activity represents nanomoles of NADPH oxidized per gram wet weight of tissue.

calculated with 6.22 mM⁻¹ cm⁻¹ used as the extinction coefficient of NADPH. All nonspecific NADPH oxidation artivity that may occur in the absence of substrate is automatically corrected for, since the solutions differ or ly in that one contains cyclohexane. Statistical comparisons of enzyme activities were performed by the paired t test.

Results

Fig. 1 shows the relationship between cytochrome P-450 activity and smooth muscle content in normal and neoplastic human uterine tissue. No apparent correlation (r = 0.34) was found between smooth muscle content and cytochrome P-450 activity in human uterus and leiomyoma. However, leiomyomas contained significantly higher cytochrome P-450 activity than adjacent mycmetrium when expressed in terms of specific activity (p < 0.01) but not in terms of total activity (p > 0.1). This result suggests that no change in cytochrome F-450 activity occurs but that levels of other proteins may be decreased in leiomyomas.

Table I summarizes cytochrome P-450 activity in normal and neoplastic uterine tissue. As previously men-

[†]One specimen was unclassified.

[‡]Specific activity ranges determined in our laboratory for untreated and phenobarbital-induced rabbit liver microsomes.

tioned, the specific activity for cytochrome P-4EO was significantly higher in leiomyomas than in normal azeri (p < 0.01). The specific activities (mean \pm SE = $\neg \epsilon$ re 2.87 ± 0.26 and 1.60 ± 0.11 nmol/min/mg f=r the leiomyomas (n = 17) and normal uteri (n = 30), $\tau =$ spectively. Interestingly both of these values were in the specific activity range (1 to 3 nmol/min/mg) of that observed for untreated rabbit liver microsome.. Furthermore, there was no significant difference with regard to cytochrome P-450 activity expressed as e-ther specific or total activity (p > 0.1 for both studies 5etween leiomyomas and myometrium in either plass of the menstrual cycle. Moreover, there was no significant difference in enzyme activity among the gyne clogic indications for hysterectomy (abnormal uterine beeding, urinary stress incontinence, dysmenorrhea, and uterine descensus).

Comment

At present, there is no evidence of cytochrom € E ≤ 50 in muscle of any type, although peripheral appratization (possibly catalyzed by cytochrome P-450) cf androgens is believed to occur in muscle.14 Furthernare, because the uterus is composed of a heterogener us cell population, the contribution of smooth muscle to the variation in the specific enzyme activity was nurstigated. Although there was no apparent correlator between cyclohexane hydroxylation activity and mooth muscle content as was demonstrated for prostaglandin receptors in myometrium,15 a significant difference (p < 0.01) in enzyme activity between leiomyomas and myometrium existed when expressed in terms of specific activity but not in terms of total activity (p > 11). This result suggests that decreased protein lev-le may be responsible for higher enzyme activities observed in leiomyomas rather than increased levels of cyto-hrome P-450.

Leiomyomas arise from either uterine or varcular smooth muscle and it has been shown that they contain less smooth muscle than normal uterine myome x2:m. 16 Recently, it was demonstrated that prostaglard n receptor (prostaglandin E: prostaglandin F_{2α}) con ⊨∎: was lower in leiomyomas than in normal myometriam and that the difference in prostaglandin receptor: in the two tissues could be accounted for by the lower smooth muscle content of the leiomyomas. 16 As a result of the low prostaglandin receptor content, it is sugges-ed that leiomyomas, compared with unaffected normal myometrium,16 are relatively insensitive to the coltabile and various other actions of prostaglandins. However, leiomyomas are known to be more sensitive to steroids (estrogen, progesterone) than normal myometrum, because of the higher estrogen and progesterone meetotor levels in leiomyomas.17,18 It is well known that exergen and progesterone receptor levels peak at midc-ce and progressively decline during the secretory phase of the menstrual cycle. ¹⁹ Since no significant difference (p > 0.1) in hydroxylation activity was observed between the two phases of the menstrual cycle (leiomyomas and normal myometria), it is likely that estrogen and/or progesterone receptors are not involved in the regulation of cytochrome P-450 biosynthesis. This has also been suggested by our recent study evaluating similar parameters in human breast cancers categorized by levels of sex hormone receptors.³

As shown in Table I, mean activities for both leiomyomas and normal uteri were within the range of untreated rabbit liver microsomes. The levels of this enzyme activity in rabbit liver determined in our laboratory ranged from 1 to 3 nmol/min/mg of microsomal protein. Activities ranged from 0.48 to 3.75 nmol/min/mg for normal myometrium and from 1.00 to 4.99 nmol/min/mg for the leiomyomas. Furthermore, all leiomyomas (n = 17) assayed as well as 26 of 30 (87%) of the normal uteri exhibited levels of cytochrome P-450 activity comparable to those of tissues (for instance, liver) actively engaged in drug metabolism.

This study indicates that leiomyomas may be a useful model for investigating the relationship between cytochrome P-450 hydroxylation of antiestrogens such as tamoxifen and the levels of estrogen receptors. We have shown that tamoxifen induces progestin as well as estrogen receptors in certain patients with endometrial carcinoma.20 However, since it is known that some endocrine-responsive carcinomas such as those of the breast may be unresponsive to tamoxifen even though the biopsy specimens contained estrogen and progestin receptors, a possibility of competition exists between enzyme and receptor for the drug. Thus we suggest that leiomyomas with high levels of cytochrome P-450 will be less responsive to an endocrine stimulus. It is also possible that these enzymes may metabolize tamoxifen to a more active analogue as has been demonstrated in the liver.8 Regardless, there is evidence that the role of cytochrome P-450-linked enzymes should be explored more fully in endometrial and breast tissues.

We greatly appreciate the assistance of Drs. James G. Kuhns and Stewart E. Wolfson of the Pathology Department of Norton's/Kosair/Children's Hospital in the collection of tissue specimens.

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Note: A complete list of references is available upon request.

Gynecologic amyloidosis

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Amyloidosis is a generalized metabolic disorder characterized by deposition of insoluble protein in the extracellular space of various organs. We have treated a woman presenting with menorrhagia and anemia, who was subsequently diagnosed as having systemic amyloidosis. This represents the first known documentation of amyloid involvement of the female reproductive tract. (AM J OBSTET GYNECOL 1985;153:555-6.)

Key words: Amyloidosis, microcytic hypochromic anemia, alopecia, macroglossia, serum immunoelectrophoresis

Systemic amyloidosis involving the female reproductive tract was the histologic diagnosis subsequent to surgical treatment of a woman presenting with menor-rhagia and anemia thought to be associated with uterine leiomyomas. We report the first known documentation of this rare medical finding.

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Case report

A 47-year-old black woman, gravida 8, para 7, abortion 1, was admitted to the Ohio State University Hospitals for evaluation of frequent, heavy menstrual bleeding. Historically, the patient noted bleeding episodes every 21 days, lasting for 8 to 10 days. These menstrual periods had remained consistent for 1 year. She denied any intermenstrual or postcoital bleeding and did not experience any vasomotor symptoms. Gynecologic examination revealed an enlarged uterus consistent with the size of a 12-week gestation. The pregnancy test was negative, and the Papanicolaou smear test result was benign. A microcytic (mean corpuscular volume, 79 μ^3) hypochromic (mean corpuscular hemoglobin, 26.2 pg) anemia was found with a

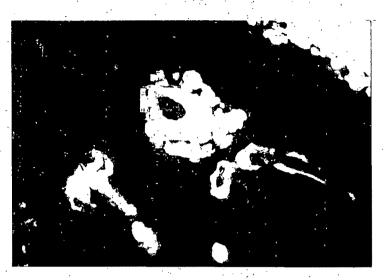


Fig. 1. Amyloid involvement of Literine blood vessels. (Thioflavin-T fluorescence stain.)

hemoglobin level of 10.9 gm/dl. Platelet cours and clotting parameters were within normal ranges

Significant past history included bilateral carpal cunnel syndrome, which was surgically treated in 975. Severe; noncicatricial alopecia developed in 19% and multiple dermatologic consultants failed to establish a specific etiology. The patient subsequently experienced dyspnea on exertion, orthopnea, and pedal ederac with established chronic hypertension. Congestive he are failure was diagnosed and appropriate therapy was instituted. Macroglossia was noted during the preoperative evaluation.

The presumptive gynecologic diagnosis was therine leiomyomas. Preoperative uterine curettage dicumented a uterine cavity depth of 11 cm. Prolimative endometrium without endometrial hyperplasia vas the histologic diagnosis. Subsequent total abdomira hysterectomy and bilateral salpingo-oophorectomy with incidental appendectomy were performed. The postoperative course was unremarkable.

Pathologic findings included a uterus of 350 pm and myometrial thickness of 4.5 cm. Histologic stuly documented extensive amyloid involvement of the userine cervix and corpus, fallopian tubes, ovaries, appendix, and omentum. These findings were confirmed by amyloid-specific Thioflavin-T fluorescence stain of all involved tissues (Fig. 1). Postoperative evaluation included a normal serum immunoelectrophoresis and quantitative immunoglobulins. Results of bone matrow examination were normal.

Comment

The preoperative diagnosis in this patient was sterine leiomyomas. Amyloidosis had not been suspected preoperatively. In retrospect, this patient had exhibited many signs and symptoms of amyloidosis before the operation. Carpel tunnel syndrome and other compressive neuropathies, alopecia, macroglossia, and car-

diomyopathies can be seen in patients with amyloidosis.

Amyloid involvement of the skin, tongue, heart, uterus, fallopian tubes, and ovaries is most consistent with a type I pattern seen in multiple myeloma or primary amyloidosis. In this case, the serum immuno-electrophoresis and immunoglobulin findings were normal, and the bone marrow showed no increase in plasma cells. Multiple myeloma was excluded and the diagnosis of primary amyloidosis was established.

Amyloidosis is a generalized metabolic disorder characterized by deposition of insoluble protein in the extracellular space of various organs. There are no prior reports of organ involvement in the female reproductive tract. There are reports of involvement of the male prostate, seminal vesicle, and testes. A "ground substance" resembling amyloid has been extracted in minute quantities from the cervix and uterus, but this appears to be a normal component and not related to the pathologic entity of amyloidosis.

Although no specific documentation can be established for the patient's amyloidosis as the cause of menorrhagia and anemia, it is certainly an attractive explanation. We have theorized that the amyloid infiltration of the uterine corpus compromised uterine contractility and led to the prolonged bleeding episodes during menstruation. We urge further investigation into the gynecologic manifestations of this rare medical condition.

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Prophylactic intrapartum amnioinfusion in patients with preterm premature rupture of membranes

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Patients with preterm premature rupture of the membranes are at increased risk to develop intrapartum variable decelerations and fetal distress. Short-term saline solution amnioinfusion may be of benefit in the treatment of variable or prolonged decelerations once they appear. In an effort to assess the benefit of prophylactic amnioinfusion, patients with preterm premature rupture of the membranes were studied during a 1-year period in a prospective randomized manner. Patients receiving prophylactic amnioinfusion had significantly decreased incidence and severity of variable decelerations in the first stage of labor (p < 0.005). In the second stage of labor, the incidence of severe (p < 0.005) and total (p < 0.001) decelerations was also decreased in the treatment group. The umbilical arterial pH at delivery was significantly lower (p < 0.001) as was the umbilical venous pH (p < 0.005) in the newborn infants of control patients compared with those of patients receiving amnioinfusion. This suggests that prophylactic intrapartum amnioinfusion is of significant benefit in reducing the incidence of variable decelerations and improving the metabolic state in newborn infants born to women with preterm premature rupture of the membranes. (AM J OBSTET GYNECOL 1985;153:557-62.)

Key words: Amnioinfusion, fetal distress, preterm premature rupture of membranes

Patients with preterm premature rupture of the membranes are at an increased risk of developing intrapartum fetal distress. Cesarean section rates for premature infants with abnormal fetal heart rate (FHR) tracings in labor have been reported to be between 8% and 20% with neonatal mortality rates as high as 35%. In this group of patients, the most frequent abnormal FHR patterns are repetitive variable decelerations presumably resulting from umbilical cord compression as a consequence of oligohydramnios.

In both animal and human studies it has been demonstrated that the frequency of induced or spontaneous variable deceleration patterns can be decreased with the infusion of saline solution into the amniotic cavity. ^{1, 5} In order to test the hypothesis that amniotic fluid replacement in early labor in patients with premature rupture of the membranes remote from term will decrease the incidence and severity of variable decelerations and improve the newborn metabolic state this prospective randomized study was performed. The impact of intrapartum prophylactic amnioinfusion on the frequency and severity of variable decelerations, perinatal morbidity and mortality, and rate of cesarean

section for fetal distress was studied in patients with preterm premature rupture of the membranes who were candidates for a trial of labor once delivery was indicated.

Material and methods

The patient population for this study group was drawn entirely from the population at Women's Hospital, Memorial Center, Long Beach, California, from March, 1984, to March, 1985. Most patients were transported from other hospitals and all patients were managed by the Women's Perinatal Group of Women's Hospital. Patients admitted with preterm premature rupcure of the membranes who were not in labor were enrolled in the study in a prospective manner if specific criteria were met and informed consent was obtained. The criterion for inclusion was spontaneous premature rupture of the membranes with a gestational age ranging between 26 and 35 completed weeks of gestation. Spontaneous premature rupture of the membranes was documented by patient history and confirmed by sterile speculum examination on admission. Premature rupture of the membranes was confirmed by the presence of pooling of amniotic fluid in the vaginal vault, Nitrazine-positive fluid, and the presence of ferning on microscopic examination. The cervix was visually inspected for dilation and cultures were obtained for documentation of gonorrhea and group B streptococcus. Internal digital examination was avoided in all patients prior to the onset of labor.

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Table I. Parameters used for grading variable decelerations in FHR tracings

Variable decelerations (level of FHR drop and duration of deceleration)					
Mild	Moderate	Severe			
<30 sec duration,	<70 bpm,	<70 bpn₌			
irrespective of level	>30<60 sec	>60 sec			
>80 bpm irre-	70-80 bpm,				
spective of duration	>60 sec				
70-80 bpm,		. ,			

Modified from Kubli et al.7

Ultrasound examination of all patients was zerformed within 1 hour of admission. Documentation of decreased or absent amniotic fluid, a vertex presentation, and no obvious gross fetal anomalies were æditional criteria for study candidates. At the time of sonographic examination an attempt was made to abtain amniotic fluid via sterile percutaneous amnicentesis if an accessible pocket of amniotic fluid was ilentified. Amniotic fluid was sent for immediate Gazm stain and culture (both aerobic and anaerobic) as well as assessment of pulmonary maturity. Pulmonary maturity was assumed with any of the following: a post-ive shake test, a foam stability index ≥0.47, a leci-h_n/ sphingomyelin ratio ≥1.8, or the presence of phosphatidylglycerol (Garite TJ, Freeman RK, Nagrotte MP. Fetal maturity cascade. A rapid and cost effective method for fetal lung maturity testing. Submittec for publication).

Estimated gestational age at the time of entry into the study was determined by the first day of the last normal menstrual period with early ultrasound confirmation when available. Ultrasound on admission was used to confirm the gestational age as accurately as possible.

The following were indications for exclusion from the study: a fetal lie other than vertex; premature below on admission; vaginal bleeding; inability to confirm premature rupture of the membranes grossly, chemically, or microscopically; previous cesarean section and no desire for a trial of labor; antepartum fetal distress; and unwillingness to participate in the study. Ne ther tocolytic agents nor glucocorticoids were used in any of the study patients. Patients who remained unce ivered following an initial continuous monitoring period of 48 hours in the labor and delivery unit had faily monitoring of FHR for 60 minutes.

The study design was explained to all patients entering the study and informed consent was obtained. Patients were then assigned in a random fashion to the group receiving prophylactic intrapartum amnio...fu-

sion or to the group serving as controls who would not receive amnioinfusion.

Indications for delivery included onset of spontaneous labor, induction of labor for a fetal pulmonary test showing maturity, a positive Gram stain of an amniotic fluid sample, and antepartum amnionitis. All patients had internal monitors placed (both fetal scalp electrode and uterine pressure catheter) as early in labor as possible. Patients in the treatment group received normal saline solution infused through a blood warmer in order to keep the infused fluid at a temperature of 37° C. The infusion was connected through the sidearm of an extension tubing placed between the fetal monitor and the internal pressure catheter. The initial rate of infusion was 10 ml/min for the first hour, and an infusion rate of 3 ml/min was subsequently maintained. If the patient experienced a sudden loss of a large volume of fluid secondary to position change or spontaneously, the infusion was again increased to the initial rate for I hour and then returned to the maintenance rate...

Maternal morbidity was measured by documenting amnionitis, postpartum endometritis, and prolonged postpartum hospitalization. The criteria for amnionitis were at least two of the following: fever ≥38° C, leukocytosis with a white blood cell count >15,000/mm³, or foul-smelling amniotic fluid with or without uterine tenderness to palpation. Endometritis was diagnosed in patients having postpartum fever >38° C on at least two occasions 4 hours apart associated with uterine tenderness and/or foul-smelling lochia.

Apgar scores were assigned by the neonatologist in attendance at delivery. Umbilical cord arterial and venous pH were measured immediately on delivery by careful aspiration of the appropriate umbilical vessel and rapid transport in heparinized tuberculin syringes to the blood gas laboratory. Gestational age estimates following delivery were performed by the attending neonatologist early in the nursery stay. Neonatal morbidity and mortality were ascertained from review of neonatal records.

Volume of infused saline solution, length of labor from the time of placement of internal monitors until complete cervical dilatation, and length of second stage of labor were all documented prospectively.

All intrapartum FHR tracings were reviewed in a blinded manner to ensure that the reviewer had no knowledge of patient's name, study group, newborn data, or neonatal outcome. The number and type of variable decelerations were determined by means of the classification of variable decelerations established by Kubli et al. (Table I).

The normal criteria for management of labor were used in assessing the fetus. All patients had electronic monitoring of the FHR. Cesarean section was per-

Table II. Maternal factors measured in patients receiving amnioinfusion and in those serving as controls (mean \pm SD)

	Amnioinfusion	Control	p Value*
Maternal age (yr)	25.2 ± 4.4	26.0 ± 5.2	NS
Parity	0.7 ± 1.0	1.1 ± 1.2	NS
Gestational age (wk)	31.1 ± 2.2	31.1 ± 2.5	NS
Length of labor (hr)†	4.1 ± 1.5	3.5 ± 1.3	NS
Antepartum hospital days	1.9 ± 1.7	2.6 ± 2.4	NS
Postpartum hospital days	2.5 ± 1.1	2.7 ± 1.4	NS

^{*}Student's t test.

formed for fetal distress in the presence of uncorrectable, persistent late decelerations, recurrent severe variable decelerations, or recurrent or uncorrectable prolonged decelerations.8 Induction or augmentation of labor was performed with intravenous oxytocin. Patients being delivered for clinical or occult amnionitis received appropriate antibiotics during and after birth. Statistical comparisons were performed by use of Student's t test and χ^2 or Fisher's exact test.

Results

Informed consent was obtained from a total of 66 patients for the study and the patients were then randomized. Sixty-one were included, 29 (48%) of whom were randomized to receive amnioinfusion and 32 (52%) of whom were randomized to the control group. Of the five patients eliminated from the study, three had a fetal presentation other than vertex at the time of labor after having a documented vertex presentation at the time of randomization. The other two patients were eliminated because of the detection of antepartum fetal distress during routine daily nonstress testing. All patients had singleton pregnancies with the exception of one set of twins. One patient desiring a trial of labor following a previous low transverse cesarean section was included. Both the twin gestation and the patient with a previous cesarean section were in the amnioinfusion group. The volume of warmed saline solution infused during birth ranged from 735 to 1650 ml with a mean of 1160 ml.

Treatment and control groups were equally matched for maternal age, parity, gestational age, length of labor, and antepartum and postpartum hospital days (Table II). Range of gestational age at delivery was randomly distributed between 26 and 35 weeks' gestation (Table III). Amniocentesis was successful in 29 patients (47%), 15 in the amnioinfusion group and 14 in the control group. A mature pulmonary cascade was documented in 13 patients (21%) and a positive am-

Table III. Distribution of patients by completed weeks of gestation in patients receiving amnioinfusion and in those serving as controls

Weeks' gestation	Amnioinfusion	Control	p Value*
26-27	3	4	NS
28-29	1	3	NS
30-31	12	8	NS
32-33	8	13	NS
34-35	5	4	NS

^{*}Fisher's exact test.

niotic fluid Gram stain was noted in eight patients (13%) (Table IV). No differences existed between the groups in the incidence of spontaneous labor, mature pulmonary tests, positive amniotic fluid Gram stain, or amnionitis prior to the onset of labor as the indication for delivery (Table IV).

Newborn data revealed no differences with respect to newborn birth weight, Apgar scores at 1 and 5 minutes, neonatal hospital days, and neonatal deaths. Although the differences were not statistically significant, there appeared to be a trend toward lower 1-minute Apgar scores in the control group (Table V). Two of the three neonatal deaths were in infants born at 26 weeks' gestation with birth weights of 730 and 800 gm. The third neonatal death occurred in a severely depressed 1500 gm infant born at 31 weeks' gestation in the control group with delivery by cesarean section for fetal distress

The incidence of variable decelerations (mild, moderate, severe, and total) was determined for both the first and second stages of labor (Table VI). Statistically significant differences were seen with respect to the incidence of mild (p < 0.005), moderate (p < 0.005), severe (p < 0.005), and total (p < 0.005) variable decelerations in the first stage of labor, with all being less in the amnioinfusion group. Significant differences were also noted in the second stage of labor for the incidence of severe (p < 0.005) and total (p < 0.001) variable decelerations. Additionally, strong statistical significance was seen in the difference of umbilical arterial pH (p < 0.001) and umbilical venous pH (p < 0.005) between the study groups (Table VII) with values in the control group being lower.

In the 61 patients studied, there were nine (15%) cesarean sections (Table VIII). In the amnioinfusion group, one patient underwent a repeat cesarean section for failure to progress in labor and one patient had a primary cesarean section for fetal distress (3%). In the control group there were seven cesarean sections for fetal distress (22%). All of these patients had recurrent severe variable or prolonged decelerations and one patient was noted to have an occult cord prolapse. Although statistical significance could not be established,

[†]Measured from the time of internal monitor placement to delivery.

Table IV. Indications for initiation of labor in patients receiving amnioinfusion and in those serving as controls (number of patients, percent of group)

	Azzioinfusion .		Control		
	ņ	%	n	. %	p Value
Spontaneous labor	Iε	62	21	64	NS*
Mature pulmonary cascade		24	6	18	NS*
Positive amniotic fluid gram stain	٤	10	5†	16	NS‡
Amnionitis prior to labor	I	3	1	3	NS‡

^{*}x2.

Table V. Newborn factors measured in patients receiving amnioinfusion and in those serving as controls (mean \pm SD)

	Amnioinfusion	Control	p =a=ce*
Birth weight (gm) Apgar score	1746 ± 509	1711 ± 494	I E
l min	7.1 ± 1.7	6.6 ± 2.2	-15
5 min	8.2 ± 1.0	8.4 ± 1.0	. 15
Neonatal hospital days	24.2 ± 18.5	21.8 ± 23.0	N3
Neonatal deaths	1	2	J.E
Low I min Apgar score (<4) (%)	3.4	13.0	75-
High I min Apgar score (>7) (%)	69.5	58.0	45-

^{*}Student's t test.

there appeared to be a clear trend (0.10 < p < DIS). Two patients developed amnionitis prior to the obset of labor, one from each group (Table V). Four patients had endometritis, one in the amnioinfusion group and three in the control group. Three of these patients had cesarean deliveries and the one patient with poster-tum endometritis following vaginal delivery was retrieventrol group.

Comment

Amniotic fluid has several distinct functions. Included among these are assistance in fetal pulnidary development, facilitation of fetal mobility, and protection of the fetus from external trauma. The presence of adequate amniotic fluid appears to minimize compression of both the umbilical cord and placenta secondary to external pressure. An association between decreased amniotic fluid volume and umbilical cord compression has been reported in antepartum heart rate testing of patients with intrauterine growthmetartation and oligohydramnios as well as during birth following artificial rupture of the membranes early in labor. This compression of the umbilical cord

Table VI. Incidence of mild, moderate, and severe variable decelerations in the first and second stages of labor (number per hour, mean ± SD)

	Amnioinfusion	Control	p Value*
First-stage labor†			
Mild	1.6 ± 1.3	4.2 ± 3.2	< 0.005
Moderate	0.6 ± 1.4	2.3 ± 2.3	< 0.005
Severe	0.2 ± 0.2	1.4 ± 1.8	< 0.005
Total	2.4 ± 2.4	7.9 ± 5.6	< 0.005
Second-stage labor			
Mild	3.6 ± 3.8	2.3 ± 4.4	NS
Moderate	5.6 ± 5.6	5.6 ± 5.9	NS
Severe `	2.9 ± 3.1	10.1 ± 7.7	< 0.005
Total	7.2 ± 7.0	18.0 ± 6.0	< 0.001

^{*}Student's t test.

blood flow through the umbilical vessels, with blood flow within the thin-walled umbilical vein being impeded before the flow in the thicker-walled umbilical arteries. Fetal compromise may result as the degree and duration of cord occlusion increase.

The characteristic changes in the FHR that accompany intermittent umbilical cord occlusion are variable decelerations.¹³ The most common type of FHR deceleration pattern seen in labor is the variable deceleration and since this pattern suggests the presence of intermittent cord occlusion, recurrent variable decelerations are a difficult pattern to manage during labor. Variable decelerations are classified by the duration and degree of FHR slowing as mild, moderate, and severe, and their frequency and severity correlate with fetal capillary pH.⁷

Fetal tolerance for recurrent variable decelerations is related to the duration of insult as well as the gestational age. Abnormalities in FHR are tolerated more poorly by both premature and low birth weight infants

[†]One patient in the control group had both a mature pulmonary cascade and a positive Gram stain. ‡Fisher's exact test.

 $[\]dagger \chi^2$; Fisher's exact test.

[†]Measured from the time of placement of internal monitors to complete cervical dilation.

Table VII. Measurement of umbilical arterial and umbilical venous pH at the time of delivery in patients receiving amnioinfusion and in those serving as controls (mean $pH \pm SD$)

рН	Amnioinfusion	Control	p Value*
Umbilical arterial	7.34 ± 0.05	7.23 ± 0.08	<0.001
Umbilical venous	7.40 ± 0.06	7.34 ± 0.06	<0.005

^{*}Student's t test

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when compared with the same FHR changes in term, appropriately grown newborn infants.3 Further, the incidence of abnormalities of the intrapartum FHR is increased in pregnancies complicated by preterm premature rupture of the membranes, with variable decelerations being the most frequent FHR abnormality. Cesarean section rates for fetal distress are increased in these patients and the incidence of neonatal mortality has been reported to be markedly increased as well.¹⁻³

Miyazaki and Taylor have reported on the apparent therapeutic utility of short-term saline solution infusion into the uterine cavity for patients in term labor experiencing repetitive variable or prolonged decelerations. Since patients with preterm premature rupture of the membranes are at an increased risk for the development of abnormal FHR patterns and additionally tolerate these patterns poorly, we elected to prospectively study the potential benefit of prophylactic amnioinfusion in this group of patients at the onset of labor, prior to the development of variable decelerations. Since all patients were studied prior to term, the infused solution was raised to body temperature in an attempt to minimize the risk of hypothermic change in the fetal environment.

Study patients were evenly matched for maternal age, parity, mean and range of gestational age, length of labor, and hospital days (Tables II and III). Newborn birth weights, Apgar scores, hospital days, and mortality were no different between the treatment and control groups (Table V). Percentage of successful amniocenteses, delivery indications (Table IV), and infectious morbidity were similar in both groups.

However, all forms of variable decelerations in the first stage of labor as well as severe and total decelerations in the second stage of labor were significantly decreased in patients receiving prophylactic amnioinfusion (Table VI). This observation accompanied the finding that the umbilical arterial and venous pH at delivery was significantly lower in the control group (Table VII). Additionally, although mean Apgar scores at 1 minute were not different, there was a greater

Table VIII. Delivery mode for patients receiving amnioinfusion and in those serving as controls

Type of delivery	Amnioinfusion	Control	p Value
Vaginal Cesarean section	27	25	NS*
Intrapartum fetal distress	1	7	NS†‡
Failure to progress	1		NS†

*χ². †Fisher's exact test. $\pm \chi^2 = 2.89$; 0.10 .

incidence of low 1-minute Apgar scores in the control group. Compared with umbilical pH, Apgar scores are much less sensitive measures of the neonatal metabolic state, particularly in the premature infant.14 It is interesting to note that although the differences in umbilical blood pH are statistically significant, the differences in umbilical arterial pH are more highly significant than the differences in umbilical venous pH. This finding is consistent with the physiologic explanation of variable decelerations as a change in FHR resulting from a vagal response to decreased blood return to the heart following occlusion of the umbilical vein.13 Interruption of well-oxygenated fetal blood will lead to a respiratory acidosis as reflected by a decreased umbilical arterial pH. If this condition is allowed to continue or worsen, a metabolic acidosis may result with both umbilical arterial and venous pH being lowered.

Although not as objective or sensitive a measure of fetal state as blood pH, the cesarean section rate for fetal distress also was increased in the control group. Primarily because of the population size of the study groups, statistical significance was not demonstrated. However, a clear trend was evident with a cesarean section rate for fetal distress of 22% in the control group compared with a rate of 3% in the patients who received amnioinfusion.

Neonatal morbidity was not significantly different between the study groups. Distribution of complications and length of stay in the nursery were essentially the same for the entire population with the main complications (respiratory distress syndrome, patent ductus arteriosus) being related to prematurity. Neonatal mortality was also no different with one potentially preventable death. This death occurred in a 1500 gm infant in the control group delivered at 31 weeks' gestation by emergency cesarean section for fetal distress.

In summary, this study has demonstrated the benefit of prophylactic amnioinfusion in patients with preterm premature rupture of the membranes in the reduction of incidence and severity of variable decelerations and in improvement of the metabolic state at delivery as

reflected by higher umbilical blood pH. The esse of administration, patient acceptance, and lack of complications make this treatment a viable clinical mediatry for these high-risk pregnancies. Caution needs to be exercised in attempting to extrapolate these rest les to term or postdates patients with oligohydramnios. These patients also seem to be at increased risk for developing intrapartum fetal distress but the FHR changes may be due to placental factors in addition to intermitten. cord occlusion.

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Is there a need for digital examination in patients with spontaneous rupture of the nembranes?

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A prospective comparison between digital and visual s== lum examination was made in 133 pregnant patients. The coefficient correlation between the two ecaminations of effacement and dilation of the cervix was 0.74. The present results suggest that cervical evaluations by means of speculum examination in the assessment of pregnant patients with spontaneous rufiles of membranes are adequate. (Ам J Obstet GYNECOL 1985:153:562-3.)

Key words: Cervical examination, premature rapture of membranes

The role of digital examination in the management of patients with premature rupture of membrines is. controversial. Some authors perform a digital =>=mination to ascertain the status of the cervix, whereas

tion may be made by means of speculum examination; however, the accuracy of this method in assessing cervical changes has not been validated. In an attempt to determine the value of a digital examination in addition to a speculum examination under these circumstances, we have assessed the correlation between speculum and

digital examinations; using cervical dilation and ef-

facement as the parameters measured. We were also

others2 do not because such examinations may increase the likelihood of chorioamnionitis. A cervical evalua-

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interested in any possible difference between examiners with different years of experience.

Methods

Obstetric patients were randomly selected from the outpatient clinics and labor admitting rooms at Loma Linda University Medical Center, Riverside General Hospital of the University Medical Center, and San Bernardino County Hospital.

The only selection criterion to enter the patient in the study was a pregnancy >32 weeks, regardless of the presence of labor, rupture of the membranes, or maternal illness. The examinations were performed by interns, residents, or attending physicians. The goals of the study were not explained to the examiners. Cervical evaluations were performed by a speculum examination followed by a digital evaluation carried out by the same individual.

Two of the investigators analyzed and coded the data. The cervical dilation was given a score of 0, 1, or 2 as follows: 0 =closed or fingertip, 1 = 1 or 2 cm dilated, $2 = \ge 3$ cm of dilation. The effacement was coded 0, 1, or 2 depending on the percentage of shortening of the cervix: 0 = 0% to 39%, 1 = 40% to 79%, 2 = 80%to 100%. The scores for dilation and effacement were summed. The total score for both the digital and the speculum examinations was used to determine correlation coefficients between the examinations and between the examiners' experience.

Results

A total of 133 patients were included in the study. The examinations were performed by interns in 56 cases, by residents in 46 cases, and by attending staff in 31 cases. Fig. 1 shows that the correlation coefficient for speculum versus digital examination was 0.72 (p < 0.001) for all examiners. The correlation by different examiners was 0.70 for the interns, 0.85 for the residents, and 0.53 for the attending staff. Those differences were not statistically significant by Fisher's Z test. (p > 0.10). Comparisons of effacement and dilitation by digital and speculum examination showed a correlation of r = 0.61 and 0.68. A symmetrical regression analysis of the data3 gave the equation y = 0.098 + 1.185x (r = 0.72, p < 0.001). This means that the correlation between both examinations was good throughout the scale with less than a 20% mean variation between digital and speculum examination, a dif-

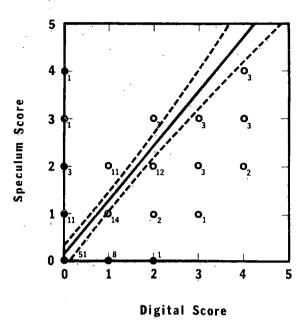


Fig. 1. The score from the digital examinations is plotted versus the score from the speculum examinations. Numbers of patients at each point are given.

ference that is not clinically significant. A review of the individual records shows that in only two patients did results of the speculum and digital examinations differ significantly enough to alter the course of management by the criteria of Duff et al.1

Comment

Man / authors have suggested that a sterile speculum examination in the evaluation of patients with ruptured membranes is sufficient; however, this technique has not been validated to the best of our knowledge. The present results indicate that cervical evaluation by speculum examination correlates reasonably well with digital examination. This suggests that cervical evaluations by means of speculum examinations should be adequate for most patients with ruptured membranes.

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Hermansky-Pudlak syndrom in pregnancy: Two case studies

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Pregnancies in two patients with Hermansky-Pudlak syndrome, oculocutaneous albinism with hemorrhagic diathesis, are presented. Outcomes were good despite prior reports of postpartum hemorrhage in patients with this syndrome. This autosomal recessive disease is relatively common in parts of Puerto Rico. Suspected patients should be screened with platelet aggregation studies. (AM J OBSTET GYNECOL 1985; 153:564-5.)

Key words: Hermansky-Pudlak syndrome, plateles, postpartum hemorrhage, albinism

Oculocutaneous albinism in association with hemorrhagic diathesis was first reported by Hermansky 17d Pudlak in 1959. The syndrome consists of tyrosin 132-positive oculocutaneous albinism, storage pool-ceficient platelets, and accumulation of a yellow grant ar substance in reticuloendothelial cells, circulating macrophages, and other tissues, including the lung. Bleeding time may be normal but becomes prolonged with aspirin ingestion. Menorrhagia and postpartum hemorrhage have been described. Hermansky-Pudlak expedrome, which is inherited as an autosomal recess we trait, has been described in kindreds from India, the Netherlands, and Puerto Rico.

Case reports

Case 1. A 16-year-old Puerto Rican primigravid adolescent presented for prenatal care at 20 weeks' gastation. Evaluation of prolonged bleeding after a tooth extraction had led to the diagnosis of Hermansky-Fudlak syndrome several years earlier. The patient also gave a history of mucous membrane bleeding and menorrhagia. Initial physical examination showed the depigmentation, blond hair, nystagmus, and poor visual acuity. Laboratory evaluation included hematorit, 41.2%; platelet count, 214,000/mm³; prothrombin time, 11.5 seconds (control, 10.5 seconds); and partial thromboplastin time, 35 seconds (control, 35 seconds). A template bleeding time was 15 minutes (normal₂ ≤9 minutes).

She presented in labor at 40 gestational weeks. Prophylactic administration of platelets resulted in a transfusion reaction, which was treated with diphenhydramine and hydrocortisone. After an otherwise unevent-

ful oxytocin-augmented labor, she was delivered of a 3500 gm male infant with Apgar scores of 8 and 9 at 1 and 5 minutes, respectively. A first-degree perineal laceration was repaired while she was under local anesthesia. There was no abnormal intrapartum or postpartum bleeding. Hematocrit on the second postpartum day was 40.1%.

Case 2. A 40-year-old Puerto Rican woman, gravida 5, para 4, presented for prenatal care at 14 weeks' gestation. Her four children had been born in Añasco, Puerto Rico. She denied any history of postpartum hemorrhage. Her medical history was notable for prolonged bleeding from minor cuts, occasional epistaxis, heavy bleeding at the time of a tooth extraction, and one episode of menorrhagia.

Upon moving to Connecticut she had developed shortness of breath and was hospitalized. A presumptive diagnosis of Hermansky-Pudlak syndrome, based on oculocutaneous albinism and a history of bleeding diathesis, was confirmed by abnormal results of platelet aggregation studies. Pulmonary function tests showed obstructive and restrictive lung disease, and chest x-ray films showed interstitial pulmonary fibrosis. She responded well to therapy with bronchodilators. Biopsy specimens of multiple actinic keratoses showed two to be basal cell carcinomas.

Physical examination at the first prenatal visit revealed pale skin, red hair, green irides, and nystagmus. Scattered wheezes were heard on chest auscultation. The size of the uterus was consistent with 14 gestational weeks. Laboratory evaluation showed hematocrit, 35.4%; platelets, 250,000/mm³; and normal prothrombin time and partial thromboplastin time. Template bleeding times varied from 6 to 15 minutes during her antepartum course.

Onset of labor was at 39 weeks' gestation. Admitting laboratory tests showed hematocrit, 33.4%; platelets, 321,000/mm³; and bleeding time, 6 minutes. The first stage of labor was uncomplicated. The second stage was complicated by epistaxis. A 3420 gm female infant with Apgar scores of 8 and 9 at 1 and 5 minutes, respectively, was delivered over an intact perineum. Estimated blood loss was 400 ml. The patient underwent a postpartum

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Reprint requests: Rosemary Reiss, M.D., Division of Maternal-Fetal Medicine, Department of Obstetrics and Grnecology, The Ohio State University, 534 Means Hall, Columbus, OH 43210. tubal ligation with minimal blood loss. At the time of discharge, the hematocrit was 29%.

Comment

Scant information is available about the obstetric course of women with Hermansky-Pudlak syndrome. The few published accounts present dire outcomes due to hemorrhagic diathesis. Verloop described a Dutch patient who suffered massive postpartum hemorrhage following each of her five deliveries.³ Gerritsen reported a similar history in patients from another Dutch kindred.² Witkop et al.² reported the death of a Puerto Rican patient due to hemorrhage following the birth of an affected daughter.²

Our experience suggests that postpartum hemorrhage may be avoided in patients with Hermansky-Pudlak syndrome if the diagnosis is known and salicylates are avoided. Our first patient's only peripartum complication was a platelet transfusion reaction. Patient 2 had no obstetric complications due to Hermansky-Pudlak syndrome, though she exhibited its dermatologic and pulmonary sequelae.

Although Hermansky-Pudlak syndrome is rare, its incidence in the population of the Arecebo-Aguadilla area of northwest Puerto Rico has been estimated at 1:2000. The kindred of patient 2 included at least four affected individuals. They estimated that Añasco, 17

kilometers from Aguadilla, included 30 albinos among its population of 5000. Of the 92 Puerto Rican albinos known to Witkop et al.³ as of 1983, 90 had Hermansky-Pudlak syndrome. Puerto Rican patients with the syndrome are less pigmented than other family members and typically have red or red-brown hair, green or hazel irides, and nystagmus.

In view of the reported hemorrhagic complications of Hermansky-Pudlak syndrome, obstetrician-gynecologists should be aware of the diagnosis. Albino patients should be screened with platelet aggregation studies. Those who have Hermansky-Pudlak syndrome should use sunscreens and avoid medications that alter platelet function. Bleeding times should guide intrapartum management. Platelet transfusions should be available but reserved for those with prolonged bleeding times or hemorrhage.

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Fetal subcutaneous scalp Po₂ and abnormal heart rate during labor

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Intrapartum fetal heart rate and subcutaneous scalp Po_2 were continuously measured with a combined electrocardiogram— Po_2 needle electrode in 34 patients. The incidence of fetal heart rate abnormalities increased significantly with decreasing subcutaneous Po_2 , from 0.8% of the 10-minute periods in which subcutaneous Po_2 was ≥ 25 mm Hg to 53% of the periods in which subcutaneous Po_2 was ≤ 10 mm Hg. (AM J OBSTET GYNECOL 1985;153:565-6.)

Key words: Fetal heart rate, hypoxia, oxygen electrode, oxygen tension

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Little is known about fetal oxygenation during normal and abnormal fetal heart rate (FHR) in the human. The development of a small electrode for continuous measurement of the Po₂ of fetal scalp subcutaneous tissue and the fetal electrocardiogram (Fig. 1), as previously described, gave us the opportunity to study fetal oxygenation during normal and abnormal FHR.

Thirty-four patients were studied, 24 of whom were

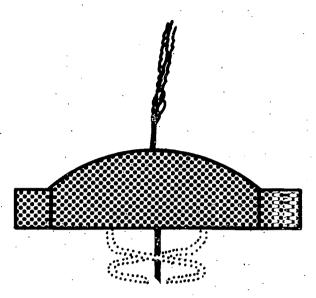


Fig. 1. The scalp electrode for recording fetal subcutaneous PO₂ and the electrocardiogram. The oxygen needle—electrode is mounted in the center of the spiral.

considered to be at high risk. Mean cervical dilezion at the time of application of the electrode was $\Sigma \subseteq cm$ (range, 1.5 to 4 cm). The recordings were contrued in all patients until delivery, except for four who were delivered by cesarean section. Mean recording time as 3.2 hours (range, 0.8 to 5.8 hours), excluding their ital 30 minutes of each recording allowed for stabilization of the Po₂ electrode. The Po₂ electrodes were calibrated before and after each experiment; when the difference exceeded 10%, the recordings were discarded.

FHR was analyzed with use of the criteria des=r bed by Hon and Quilligan,² but baseline FHR varātēlty was not taken into account. The following FH3 patterns were classified as abnormal: moderate and exerciant exerciant decelerations, all types of late decelerations and baseline tachycardia (>180 min⁻¹), and bradycardia (<100 min⁻¹). For comparison of FHR and subcutaneous Po₂, all recordings were divided into periods of 10 minutes. Once an abnormal FHR pattery occurred during a 10-minute period, this period was classified as abnormal. Fetal subcutaneous Po₂ for a 10-minute period was calculated as the mean of submanneous Po₂ values read at 1-minute intervals, and subsequently classified in one of the following ranges: = 0, 10 to 14, 15 to 19, 20 to 24, and ≥25 mm Hg.

The incidence of FHR abnormalities increase a significantly with decreasing subcutaneous Po₂, from \$8% of the 10-minute periods in which subcutaneous Po₂ was 25 mm Hg or higher to over half (52%) of the periods in which subcutaneous Po₂ was below 10 mm Hg (Table I).

Transient variations in subcutaneous Po₂ assecuted with uterine contractions were commonly observed, with the most consistent pattern being a small initial

Table I. Relationship between subcutaneous Po₂ and FHR*

Subcutaneous PO ₂ (mm Hg)	n Periods	n Periods with abnormal FHR	%
≥25	118	1	0.8
20-24	177	.20	11.3
15-19	116	32	27.6
. 10-14	50	20	40
<10	93	48	52

*There is a significant increase in abnormal FHR with falling subcutaneous Po_2 ($\chi^2 = 79.9$, df = 4, p < 0.001).

rise in PO₂ during the first half of a contraction, followed by a subsequent fall in subcutaneous PO₂ below the level prior to that contraction. In general, these variations in subcutaneous PO₂ were observed during normal FHR patterns, as well as during abnormal ones, and the transient fall in PO₂ did not exceed 5 mm Hg. However, severe variable and late decelerations of FHR and baseline bradycardia were usually accompanied by a temporary fall in subcutaneous PO₂ of more than 5 mm Hg, and under these circumstances, subcutaneous PO₂ sometimes approached zero.

The present study indicates that subcutaneous Po₂ levels over 20 mm Hg are seldom (7%) associated with abnormal FHR patterns, in contrast to lower subcutaneous Po₂ values (38.6%), and that abnormal FHR patterns indicate low subcutaneous Po₂. Similar findings have been reported with the transcutaneous Po₂ electrode.³ The transient fall in subcutaneous Po₂ associated with uterine contractions was described in an earlier report, and is most likely the result of a reduction in placental oxygen transfer. Late decelerations were accompanied either by a greater transient decline or by a constant low subcutaneous Po₂ level. These findings are in agreement with experimental studies' which showed that late decelerations are the result of hypoxemia.

We are indebted to the staffs of the delivery suites for their helpful cooperation.

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Fetal acoustic stimulation testing: A retrospective experience with the fetal acoustic stimulation test

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The nonstress test is of accepted value in the surveillance of high-risk pregrancies. In order to improve the efficiency of testing, the authors retrospectively evaluated the adjunctive use of acoustic stimulation testing (FAS-TEST) in 1241 patients who underwent 3464 antepartum fetal heart rate tests. The results were compared to those in the previous 6 months during which time 1307 patients underwent 3573 nonstress tests. The frequency of nonreactive tests was 12.6% in the nonstress test group and 6.1% in those patients who underwent the FAS-TEST. The antepartum fetal death rates were not significantly different when the two groups were compared. The FAS-TEST decreases the percentage of nonreactive tests in a high-risk population and, as a consequence, may reduce the testing time. (AM J OBSTET GYNECOL 1985;153:567-8.)

Key words: Fetal heart rate, antepartum testing, acoustic stimulation

Antepartum fetal heart rate testing, specifically nonstress testing, is of accepted value in the antenatal surveillance of high-risk pregnancies. Two inconveniences encountered when evaluating pregnancies by means of nonstress testing are (1) a prolonged test time of possibly ≥40 minutes and (2) a falsely nonreactive test due to the state of fetal arousal. Any approach which would shorten the testing time and minimize these inconveniences would be advantageous to the patients and to those who provide care. Acoustic stimulation of the fetus has been suggested as an adjunct to improve the efficiency of antepartum fetal heart rate testing.¹

From July through November, 1984, the fetal acoustic stimulation test (FAS-TEST) was used adjunctively in the antepartum fetal testing unit at our institution. High-risk gravid women were monitored by Doppler technique to establish a baseline fetal heart rate (FHR). If after 10 or 15 minutes, fetal movement and/or FHR accelerations were not observed, acoustic stimulation of the fetus was instituted. A model 5C Electronic Artificial Larynx (EAL; Western Electric, Bell Telephone) was applied to the maternal abdomen overlying the fetal vertex. This device was described by Birnholz and Benacerrof for research on fetal hearing. Stimulation from 1 to 5 seconds was accomplished. Sound pressure levels, measured at 1 M in air, averaged 82 dB. Spectral analysis of the EAL revealed a fundamental frequency

Table I. Antepartum fetal heart rate testing, effect of adjunctive acoustic stimulation (FAS-TEST)

	January-June, 1984	July-November, 1984*
Tests performed	3573	3464
Fatients tested	1307	1241
Nonreactive tests	452 (12.6%)	212 (6.1%)
Nonreactive test repeated	156 (34.5%)	50 (23.6%)
Persistently nonreactive test	43 (27.6%)	12 (24%)
Antepartum fetal death rate/1000	0.84	1.15†

^{*}Adjunctive acoustic stimulation used.

of approximately 80 Hz, and harmonics that ranged from 20 to 9000 Hz were also present.

A reactive test was defined as the presence of two or more accelerations of the FHR of at least 15 bpm that lasted for at least 15 seconds in a 10-minute window. If after 40 minutes, criteria for a reactive nonstress test were not met, the patient was managed according to existing protocol.

Table I shows a comparison between two periods of time, the first without and the second with adjunctive acoustic stimulation. The indications for testing were similar in the two groups. There was a 48.4% reduction in the rumber of nonreactive tests. The percentage of tests repeated was reduced, but by a smaller amount. The percentage of persistently nonreactive tests appeared to remain constant. The comparison of the antepartum death rates (within 7 days of a reactive test) revealed no significant difference between the two groups.

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 $[\]dagger \chi^2 = 0.005$, p > 0.1.

These data compare favorably with those reported by Serafini et al. who, in a nonrandomized fastion, contrasted the application of acoustic stimulation with the nonstress test. They found no significant difference between the two testing groups in the incidences of intrapartum fetal death, low Apgar scores, antepartum death, and fetal deaths.

Although retrospective in nature, our prelimmery data indicate that the FAS-TEST decreases the percentage of nonreactive tests in a high-risk population. The exact mechanism for this effect is unknown, but may be related to changes in the behavioral state of the fetus. There was no apparent change in the predictive value of a FAS-TEST compared to the standard mon-

stress test. Based on these preliminary observations, a prospective randomized clinical investigation is currently underway.

The FAS-TEST will be evaluated with regard to (1) predictive reliability, (2) the incidence of nonreactive tests, and (3) the average time to achieve a reactive test.

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Serum unconjugated estriol level as a predictor of pulmonary maturity

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Lack of respiratory distress syndrome in infants delivered ≡fer the maternal serum estriol value attained ≥15 ng/ml was investigated. In 91 cases after 34 weeks' ≡station no lecithin-sphingomyelin ratio <2.0 was found where the serum estriol level was ≥15 ng/ml end no respiratory distress syndrome in the patients delivered ≤3 days from amniocentesis. (AM J CB===T GYNECOL 1985;153:568-9.)

Key words: Estriol, lecithin-spingomyelin ratio, esziratory distress syndrome, pulmonary maturity

Maternal serum unconjugated estriol levels have been used to indicate fetal well-being. Buster at al. demonstrated in normal pregnancies a characterization rise or surge in serum unconjugated estriol levels at 35 to 37 gestational weeks. Furthermore, serial is riol levels were used to predict the gestational age of the pregnancy. This rise or surge is believed attributable to accelerated steroidogenesis in the fetal adrenal contained normally occurring at this time in gestation. With this increased steroid production and thus increasing fetal exposure to cortisol, there is thought to be a sem far increase in pulmonary surfactant production. Cin cal

experience at our institution had shown that no infant born to a mother with a serum unconjugated estriol level > 15 ng/ml, the approximate mean level indicating term, has developed idiopathic respiratory distress syndrome. These observations, coupled with the observation that the serum estriol surge always follows chronologically the rise in pulmonary surfactant, led us to investigate the relationships between the serum unconjugated estriol value and pulmonary maturity as reflected by the lecithin-sphingomyelin (L/S) ratio and the incidence of respiratory distress syndrome.

Patients undergoing amniocentesis between September, 1982, and February, 1984, at Madigan Army Medical Center for obstetric indications who had a serum unconjugated estriol level determined before or within 24 hours after the amniocentesis were included in the study. Estimated gestational age at amniocentesis was determined by last menstrual period, clinical landmarks, and serial ultrasonograms when available.

Serum unconjugated estriol levels were determined

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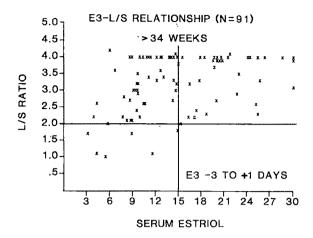


Fig. 1. Relationships between L/S ratios and serum unconjugated estriol values obtained at the time of amniocentesis after 34 weeks' gestation.

with the Estriol-Squibb Radioimmunoassay kit. The L/S ratios were determined by the chromatographic method of Borer and Gluck.

Respiratory distress syndrome was defined as the typical clinical and x-ray picture combined with the requirement for supplemental oxygen as determined by the neonatologist.

A total of 115 cases were collected in which the estriol level was determined between -3 and +1 days of the amniocentesis or the serum estriol value was ≥15 ng/ ml at any time prior to the amniocentesis. In 43 patients an estriol value of ≥15 ng/ml and an L/S ratio of ≥2.0 was found. There were 50 patients with an estriol value <15 ng/ml and an L/S ratio of >2.0 and 22 patients with an estriol value <15 ng/ml and an L/S ratio <2.0. No patients had an estriol value of ≥15 ng/ml and an L/S ratio <2.0.

Where the test might be expected to be most valid (≥34 weeks), 91 patients met selection criteria (Fig. 1). No patient with an estriol level of ≥15 ng/ml had an L/S ratio of $\leq 2.0/1$. This relationship is significant (χ^2 test; p < 0.05). In 80 cases, in which patients were delivered of infants within 3 days of amniocentesis, no respiratory distress syndrome occurred with a maternal estriol level of ≥15 ng/ml. There were five cases of respiratory distress syndrome, and in all the maternal estriol values were ≤8.9. However, in five of eight with levels of <6 ng/ml and 17 of 19 with levels of 6.0 to 9.9 ng/ml no respiratory distress syndrome occurred.

Analysis of relationships between estriol values of

≥15 ng/ml at ≥34 gestational weeks and prediction of an L/S ratio ≥2.0 demonstrated a sensitivity of 45.9%, specificity at 100%, and test efficiency of 49.5%. For predicting the lack of respiratory distress syndrome beyone 34 gestational weeks the sensitivity was 40%, the specificity was 100%, and the efficiency was 43.75%.

Within the limits of the data presented, an unconjugatec serum estriol level of >15 ng/ml is always associated with an L/S ratio of ≥2.0 or the lack of respiratory distress syndrome, or both.

An apparent lack of sensitivity in the test exists, since nearly 50% of the cases had an estriol level of <15 ng/ ml and an L/S ratio of ≥2.0. Much of this has to do with the defined level of maturity (15 ng/ml) and the reasons for this definition. An estriol level of 15 ng/ml indicates a pregnancy of 36 weeks or more and is near the mean value for 38 weeks. The level chosen must be significantly greater than values before the surge, considering biologic and assay variations. In addition, there is a wide range of normal values for a given gestationa. age and perhaps 16% of pregnancies2 never reach an estriol value of 15 ng/ml.

Lack of sensitivity could also be related to the increased number of high-risk pregnancies (four with intrauterine growth retardation and 25 with pregnancy-induced hypertension). These groups with potential placental compromise requiring amniocentesis may have low estriol values or have decreasing estriol values and a mature L/S ratio at >39 to 40 weeks. Serial values night indicate chronically low or falling estriol

Estriol determinations probably can be useful in patients who either cannot or will not have amniocentesis, are between 37 and 39 estimated weeks of gestation, and need some indication of pulmonary maturity before delivery. Perhaps further study would indicate a lower level of estriol that would increase sensitivity without losing specificity.

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Electronic fetal monitor pazed by maternal implanted pacemaker

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An implanted maternal pacemaker was found to produce ≡ stronger fetal scalp signal than did the fetal R wave, thus causing the fetal electronic intrapartum moretor o record maternal heart rate. The newborn infant was healthy and well. (AM J OBSTET GYNECOL 195:1₹3:570-1.)

Key words: Implanted maternal pacemaker, fetal heart rate, electronic monitoring

In 1964, Shouse and Acker¹ described a pregnant woman with an implanted cardiac pacemaker who had an essentially normal pregnancy and delivery. Facemakers have since been widely used in pregnant women with symptoms of heart block.² There has been one previous case reported from Sweden in which the salp electrode recorded the pulse of the implanted material pacemaker rather than the fetal R wave.³ Reported here is another case in which the activity of the material pacemaker drove the electronic fetal hear rate monitor.

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Case report

N. S., a 19-year-old woman, para 0-0-0-0, had a pace-maker implanted at age 15 because of advanced second-degree atrioventricular block and ventricular ectopy. She had no further syncopal symptoms subsequent to the implantation of the pacemaker, and the ventricular ectopy resolved. Her pregnancy was essentially uncomplicated, with her pacemaker set at approximately 70 bpm.

She was admitted to the hospital in late labor at approximately the forty-second week of pregnancy, with the cervix completely dilated and the vertex at a +2 station. There was an excessive show of blood and heavy meconium staining. The fetal heart rate could not be accurately tracked (E of Fig. 1) with the external ultrasonic transducer. An electrode was placed upon the fetal scalp under direct vision. A fetal heart rate that varied between 60 and 68 bpm was recorded with the internal monitor (I of Fig. 1). The rate was synchronous with maternal heart rate. The scope of the monitor

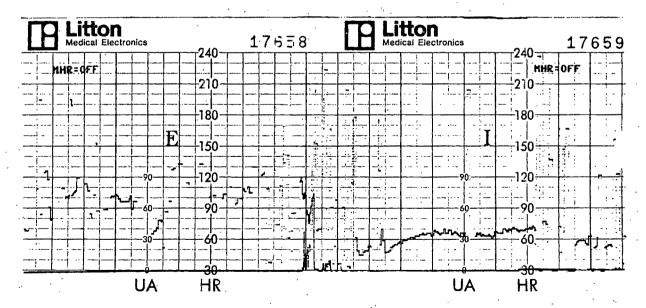


Fig. 1. Brief fetal heart rate recording bearined during second stage of labor. E refers to recording with external ultrasound transducer, and I refers to recording from fetal scalp electrode.

showed that the pulse of the pacemaker was at a higher voltage than the fetal R wave. A low forceps delivery was accomplished, and the immediate heart rate of the newborn infant was above 100 bpm. There was no meconium below the infant's vocal cords, and the placenta showed an adherent blood clot that covered approximately 15% of the maternal surface of the placenta. The scalp electrode was inserted on the scalp, and the infant did well. The maternal electrocardiographic recording from a single chest lead suggested normal function of the implanted pacemaker.

Comment

When the electronic fetal monitor records the maternal electrocardiogram from the fetal scalp electrode, it usually means that the fetus is dead. Electronic fetal monitors pass the fetal scalp signal through a band-pass filter, but they usually will select the maternal R wave for counting rather than the fetal signal if it has the highest voltage. In this case, the apparently normal

functioning maternal pacemaker transmitted its pulse at a higher voltage than the fetal R wave to the fetal scalp. The clinical significance is that cases in which there is a maternal pacemaker can be the exception to the rule that the fetus is "dead" when the maternal heart rate is recorded by the fetal scalp electrode. The literature suggests that, even though the maternal heart rate is relatively fixed in many of these cases of maternal attrioveratricular block, in the absence of severe maternal syncopal symptoms, the fetal outcome is good.²

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Thrombosis due to permanent pacemaker and oral contraceptives

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Upper-extremity deep venous thrombosis, an uncommon complication of oral contraceptive therapy, has not been described in patients with permanent pacemakers. The following case, clearly related to initiation of oral contraceptive therapy, points out perhaps another small but significant patient population in which this method of birth control is contraindicated. (AM J OBSTET GYNECOL 1985;1=3:571-2.)

Key words: Thrombosis, oral contraceptives, permanent pacemaker

Deep venous thrombosis of pelvic and lower-extremity veins is a well-recognized complication occurring in a small number of women on a regimen of oral contraceptive therapy. Thrombosis of upper-extremity veins occurs rarely and accounts for only 1% to 2% of all patients with deep venous thrombosis. "Upper-extremity venous thrombosis occurring at a pacemaker insertion site in patients using oral contraception has not, to our knowledge, been reported.

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Cas∈ report

A 22-year-old nulliparous woman was diagnosed as having sick sinus syndrome in September, 1982. She had been treated, without complication, by a demand pacemaker placed via the right subclavian vein. In September 1984, she presented 2 weeks after being placed on a regimen of oral contraceptive therapy (Nordette, 0.15 m= of levonorgestrel and 0.03 mg of ethinyl estraciol) with a 1-week history of progressive right upper-extremity nonpitting edema, cyanosis, tenderness, aching, and swelling above the right breast. There was no past history of phlebitis. She denied any history of trauma and did not smoke cigarettes. Physical examination revealed marked swelling over the right anterior chest wall above the breast, extending from midline to axilla and including the right arm. A palpable, tender avillary vein and a right subclavian vein demand pacemaker vere also noted. Following evaluation, oral contraceptives were discontinued and heparin therapy Assinitiated for presumptive diagnosis of subclavian and axillary vein thrombosis. Within 4 days her symptoms had completely resolved and she has remained asymptomatic for 2 months.

Comment

Deep venous thrombosis of the upper extremity has been documented occasionally as a result of local acute trauma. Many cases are believed to be of the "effort thrombosis" type (Paget-Schroetter syndrome). It is others are believed to be related to thoracic outlet syndrome with initial extensive compression of the subclavian vein as it passes between the first rib and clavidle, causing chronic trauma to the vein wall.

The triad of nonpitting edema, aching pain, and mattled cyanosis is present in most cases of deep versus thrombosis of the upper extremity. Easy arm faigability with activity, intermittent tingling, and versus prominence of the upper arm are often seen. One thard of patients have a palpable venous cord in the axida, upper arm, or supraclavicular area. Contrast verography is the most reliable diagnostic test used to denonstrate the site of thrombosis and its extent. Most cases involve partial venous occlusion; thus, although Depoler studies are helpful, they may not be diagnostic to

Heparin therapy, elevation of the extremity, heat, and rest are recommended. In some studies mild re-

sidual chronic claudication has been noted in as many as 65% to 75% of patients managed by medical therapy without surgical thrombectomy. To date, neither anticoagulant medical therapy nor surgical therapy has proved superior in decreasing long-term morbidity or mortality. 1.2

On the basis of observations made in this case, we believe that oral contraceptive therapy is contraindicated in women with permanent venous pacemakers. If alternate methods of birth control are unsatisfactory, the patient must be well educated in the signs, symptoms, and need for immediate evaluation of suspected venous thrombosis. Intrauterine contraceptive devices may not be a viable alternative because of the potential risk of septic embolization. For these special patients, we recommend thorough education in the proper use of a barrier contraceptive method together with contraceptive foam. This approach, along with early pregnancy termination for method failures, appears to be the safest mode of contraception in this select group of patients.

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Relationship of uteroplacental blood flow to placental clearance of maternal plasma C-19 steroids: Evaluation of mathematical models

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The concept that the placental clearance of maternal plasma dehydroepiandrosterone sulfate through estradiol formation is a function of uteroplacental blood flow in women has been disputed. We obtained data on the clearance of maternal plasma dehydroepiandrosterone through placental estradiol formation in the baboon and used these data to evaluate some mathematical models of placental clearance. Our evaluation shows that the placental clearance of dehydroepiandrosterone is proportional to uteroplacental blood flow in the baboon. (AM J OBSTET GYNECOL 1985;153:573-5.)

Key words: Uteroplacental blood flow, placental clearance, dehydroepiandrosterone sulfate, dehydroepiandrosterone

Studies of the metabolic clearance rate of dehydroepiandrosterone sulfate and the clearance of maternal plasma dehydroepiandrosterone sulfate through placental estradiol formation in pregnant women led to the hypothesis that the placental clearance of dehydroepiandrosterone sulfate is a function of uteroplacental blood flow.^{1, 2} Clewell and Meschia³ tested this hypothesis using a nonlinear mathematical model relating uteroplacental blood flow (F) to the observed clearance (Cobs) of maternal plasma dehydroepiandrosterone sulfate through estradiol formation by the placenta. Their equation

$$C_{obs} = F (1 - \exp(-C/F))$$
 (1)

also contains the parameter C, which they identify as the total placental clearance rate of maternal plasma dehydroepiandrosterone sulfate through all routes of metabclism. This equation cannot be used to estimate F from C_{obs} without also determining C. In testing the validity of the hypothesis, C_{obs} was first assigned a previously published mean value (19.3 ml/min) for the placental clearance of maternal plasma dehydroepian-drosterone sulfate formation through estradicl formation in normal, primigravid women at term, and C was calculated over a wide, arbitrarily chosen range for F. Then, with the use of the best estimate of C (19.7 ml/min), F was evaluated for a wide range of C_{obs}. Since some values obtained for F were unrealistically low, it was concluded that C_{obs} was not related to uteroplacental flow.³

In turn, the assumption on which the mathematical analysis was based, that Cobs and C are independent and do not vary directly with one another, was questioned by Everett et al.4,5 It was argued that it is likely that C and Cots are closely related and that Cots is related to F. A subsequent effort to evaluate the relationship between Cobs and C experimentally did support the contention of a direct relationship but was inconclusive in that the difference between the metabolic clearance rate of dehydroepiandrosterone sulfate immediately prior to and after delivery (used to estimate C) was actually lower than the corresponding calculated value of Cobs in about half of the patients studied.4 More recently, Fritz et al.6 measured the Cobs of maternal plasma dehydroepiandrosterone through placental estradiol formation at three levels of distal aortic blood flow (Qda)

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Table I. Distal aortic blood flow (Q_{th}) and the placental clearance of maternal plasma dehydroepiandrosterone through estradiol formation (C_{th}) in four baboons (Papio anulis)

Animal No.	Q_{da}^* (ml/min)	C _{ob} * (ml/min)
10788	550 200 320	66.8 23.6 46.3
10789	275 125 225	27.0 11.7 20.7
10795	275 140 250	42.4 22.2 35.7
10796	325 125 230	49.1 18.1 39.8

* Q_{da} was measured by an electromagnetic flowmeter C_{obs} was calculated from the product of the metabolic clearance rate of dehydroepiandrosterone and the fraction of dehydroep androsterone clearance that occurs specifically in the placent via conversion to estradiol. The metabolic clearance rate of dehydroepiandrosterone and the fraction of dehydroepiancrosterone clearance that occurs in the placenta by conversion to estradiol were determined at a steady state, following continuous intravenous infusion of tritium-labeled dehydroepiandrosterone and carbon 14-labeled estradiol to the animal.

in each of four pregnant baboons and observed that C_{obs} remained a constant fraction of Q_{da} in each animal at all flow levels. We used these data (Table I) to compare the ability of the Clewell-Meschia model and linear models to relate the C_{obs} of dehydroepiandrosterons to uteroplacental blood flow in baboons.

Since Fritz et al. 6 measured distal aortic blood $\pm w$ (Q_{da}) at the level of the maternal aortic bifurcation we made the assumption that F is a fixed proportion (k) of Q_{da} and substituted kQ_{da} for F in equation 1. Therefore, for the ith flow rate in the jth baboon,

$$C_{obs_{ij}} = k_{j}Q_{da_{ij}} (1 - exp (-C_{j}/(k_{j}Q_{da_{ij}}))) + \varepsilon_{ij}$$
 (2)

where the ϵ_{ij} are statistical error terms. We used the least-squares algorithm of Marquardt⁷ programmed in BASIC on a Tektronix 405.1 microcomputer to fit this and subsequent models to the data of Table I. Resicual sums of squares and degrees of freedom for each of the models analyzed by this method are shown in Table II.

Equation 2 allows for a unique value of C and z to be computed for each animal. A comparison of the sums of squares in Table II shows that equation 2 Ξ ts the data significantly ($F_{st} = 32.636$; degrees of freedom = 7, 4; p < 0.01). Restricting the model to a single value of C

$$C_{obs_{ij}} = k_j Q_{da_{ij}} (1 - exp (-C/(k_j Q_{da_{ij}}))) + \epsilon_{ij}$$
 (3)

does not significantly increase the residual sum: of

Table II. Residual sums of squares and degrees of freedom after fitting of the models to the data

Model	Residual sums of squares	Degrees : of freedom
Mean.	2785.94	11
Nonlinear		
Equation 2	47.94	4
Equation 3	48.77	7
Equation 3 with single k	401.35	10
Linear		
Multiple a, multiple b*	66.178	4
Single a, multiple b	69.554	7
Zero a, multiple b†	71.238	8
Multiple a, single b	97: 4 06 ·	· 7
Single a, single b	430.977	10
Zero a, single b	434.809	11

*a = Intercept; b = slope. †Equation 4 in text.

squares ($F_{st} = 0.023$; degrees of freedom = 3, 4; p.> 0.50). Further restriction of the model to a single value of k reduces the fit to the data (F_{st} = 16.867; degrees of freedom = 2, 7; p < 0.01). The values of the parameters of equation 3 resulting in the best fit to the data of Table I are C = 165 ml/min (SE = 38, degrees of freedom = 7) and the following values of k: animal 10788, k = 0.138; animal 10789, k = 0.096; animal 10795, k = 0.152; animal 10796, k = 0.163; average k = 0.137. The fact that a single k value gives a significantly poorer fit to the data than multiple values of this parameter indicates that animals differ in their values of k. This could be due to variations in the animals themselves or in the placement of the blood flowmeter transducer. We conclude that the Clewell and Meschia equation provides a satisfactory explanation of the variation in the placental clearance of maternal plasma dehydroepiandrosterone through estradiol formation as a function of Q_{da} in the baboon ($R^2 = 0.98$).

We found that in the baboon C is much larger than normal physiologic values of Cobs, in contrast to Clewell and Meschia's estimation of C for dehydroepiandrosterone sulfate in the human. When this variation occurs, the Clewell and Meschia equation converges to the prediction that Cobs is linearly proportional to uteroplacental blood flow. We therefore applied six different linear regression models to the baboon data of Table I and compared the best fitting of these models to the Clewell and Meschia curve. The six models were obtained by fitting the intercepts to zero, a single common value, or a unique value for each animal and requiring all slopes to be parallel or allowing a unique slope for each animal. Residual sums of squares and degrees of freedom for these six models are listed in Table II. The model with zero intercepts and a unique slope for each animal,

$$C_{obsij} = b_j Q_{daij} + \epsilon_{ij}$$
 (4)

results in the best fit as measured by the lowest residual mean square. No significant improvements in fit were obtained by adding an intercept ($F_{st} = 0.159$; degrees of freedom = 1, 7; p > 0.50) or intercepts (F_{st} = 0.076; degrees of freedom = 4, 4; p > 0.50) to this model, but the multiple slope model (equation 4) fits the data significantly better than the single slope, zero intercept model ($F_{st} = 13.610$; degrees of freedom = 3, 8; p < 0.01). Hence C_{obs} does appear to vary directly with Q_{da}, although the constant of proportionality, b, differs among animals. This constant has the following values: animal 10788, b = 0.126; animal 10789, b = 0.095; animal 10795, b = 0.150, animal 10796, b = 0.157; average b = 0.132. Since C_{obs} is determined so that it reflects flow through the placenta, the bi resemble the k_i of equation 3 in representing the proportion of measured blood flow reaching the placenta. The best version of the Clewell and Meschia model, equation 3, does fit the baboon data of Table I better than the strictly proportional relationship of equation 4, but the difference is not large enough to have confidence in that conclusion ($F_n = 2.523$; degrees of freedom = 1, 8; p > 0.10).

In summary, placental clearance of dehydroepiandrosterone through estradiol formation is proportional to uteroplacental blood flow in the baboon. This finding differs from Clewell and Meschia's conclusion that the placental clearance of dehydroepiandrosterone sulfate through estradiol formation is not flow-dependent in pregnant women. The most likely explanation for this discrepancy is the high placental clearance/placental blood flow ratio of dehydroepiandrosterone in the baboon (approximately 50/50 = 1.0) compared to the low clearance/flow ratio of dehydroepiandrosterone sulfate in the human (approximately 20/500 = 0.04). Thus steroids such as dehydroepiandrosterone and androstenedione (which is rapidly cleared in pregnant woment) are likely to be more sensitive indicators of uteroplacental blood flow than the more slowly cleared dehydroepiandrosterone sulfate.

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Effect of calcium supplementation on the vascular sensitivity to angiotensin II in pregnant women

Noriyoshi Kawasaki, M.D., Kazuo Matsui, M.D., Yasaharu Ito, M.D., Toshimitsu Nakamura, M.D., Toshimitsu Nakamura, M.D., Toshimitsu Nakamura, M.D., Hidetaka Ushijima, M.D., and Masao Maeyama, M.D.

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Pregnant women destined to develop pregnancy-induced 1 pertension lose refractoriness to the pressor effects of infused angiotensin II. The effect of calcium supplementation on the vascular sensitivity to angiotensin II was investigated in pregnant women. W∈ a⇒ninistered orally 600 mg of calcium L-aspartate daily to 22 pregnant women from 20 weeks of gestation to delivery. The values for the effective pressor dose of angiotensin II in the calcium-supplemented womer were compared with those in 72 nonsupplemented pregnant women. The vascular sens iun was significantly decreased after calcium supplementation. The values for the effective pressor do so of angiotensin II in the calcium-supplemented patients were 18.1 ± 1.2 ng/kg/min at 20 weeks of gesetin, 32.2 ± 2.6 ng/kg/min at the twenty-sixth week, 41.1 ± 3.4 ng/kg/min at the thirtieth week, and 25 € ± 2.9 ng/kg/min at the thirty-sixth week (mean ± SEM), while those in the nonsupplemented prti∋rds were 17.3 ± 1.2, 17.7 ± 1.6, 17.6 ± 1.2, and 15.0 ± 1.6 ng/kg/min, respectively. Assessment of the changes in the effective pressor dose of angiotensin II in the individual patients indicated that the percentile changes from 20 weeks of gestation in the calcium-supplemented patients were also significanily greater than those in 22 nonsupplemented patients. These findings suggest that calcium supplements on tends to reduce the vascular sensitivity in pregnancy. The present dosage of calcium did not affect he blood chemical parameters and did not reduce the blood pressure. The incidence of pregnancy-ir duced hypertension in the calcium-supplemented patients was 4.5%, which was smaller than that (21.2% in the nonsupplemented patients. Although there is no clear explanation of the mechanisms involved in such an effect of calcium, the present results do provide evidence to support the idea that oral calcium at a can prevent the onset of pregnancy-induced hypertension. (AM J OBSTET GYNECOL 1985;153:576-82...

Key words: Calcium supplementation; calcium, dietary; vascular sensitivity; angiotensin sensitivity test (AST); pregnancy-induced hyperension (PIH); preventive medicine

The etiology of pregnancy-induced hypertension remains obscure. Treatment has been empirical and one only known cure is termination of pregnancy. Hevertheless, the discovery of means of preventing the fevelopment of pregnancy-induced hypertension is expected.

We previously reported that normal pregnance is associated with a loss of vascular responsiveness to the pressor effects of infused angiotensin II and confirmed the findings of other workers. 2-4 Such refractorine to to angiotensin II can be seen as early as midpregnarcy. In contrast, women who are destined to develop pregnancy-induced hypertension reveal an increased sensitivity to infused angiotensin II several weeks before

the onset of the first clinical symptoms.⁴ Several investigations have suggested that the angiotensin II sensitivity test is available to screen for pregnancy-induced hypertension.^{2, 5} Although the reasons for the decreased vascular reactivity to angiotensin II in normal pregnancy and for the loss of such refractoriness in women destined to develop pregnancy-induced hypertension are not yet clearly understood, it seems that the loss of vascular refractoriness to angiotensin II may play one of the most important roles in the pathogenesis of pregnancy-induced hypertension.

It has been reported epidemiologically that the incidence of eclampsia in populations with a low calcium intake was high, whereas populations with a high calcium intake, such as the Guatemalan and Ethiopian populations, demonstrated a low incidence of eclampsia.⁶ It has also been reported that the blood pressure in pregnant women can be reduced by oral calcium administration.⁷

The aims of the present study were to evaluate (1) the effect of calcium supplementation on the vascular

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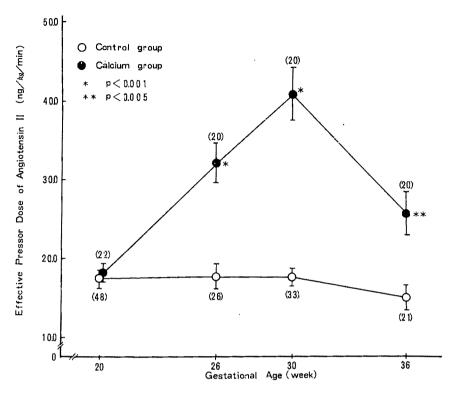


Fig. 1. Values for the effective pressor dose of angiotensin II in the control group (0) (n = 72) and the calcium-supplemented group (•) (n = 22) during pregnancy. The values are expressed as the means \pm SEM. Significant differences compared to the control group: * = p < 0.001; ** = p < 0.005.

sensitivity to angiotensin II and (2) the preventive role of calcium in pregnancy-induced hypertension.

Material and methods

Twenty-two women who were chosen from among the outpatients of the obstetric clinic of Kumamoto University Medical School Hospital were supplemented with calcium tablets every day after an initial angiotensin II sensitivity test at 20 weeks of gestation until delivery. Informed consent for the present study was obtained from all participating patients before entry. The mean age of the group, which included 10 primiparous women, was 28.2 years (range 21 to 39 years). As a control, 72 pregnant women who had not taken any calcium tablets were chosen from among the outpatients. The mean age of this group, which included 53 primiparous women, was 28.3 years (range 18 to 38 years). They did not receive any medication except for ferrous tablets for anemia. Placebo tablets were not administered for ethical reasons.

The diagnosis of pregnancy-induced hypertension was made on the basis of a blood pressure of ≥140/90 mm Hg or an increase of 30 torrin systolic blood pressure or 15 torrin diastolic blood pressure above that present earlier in pregnancy; the increase in blood pressure must have been present on at least two occasions 6 or more hours apart.

Calcium supplementation with 600 mg of calcium Laspartate (156 mg of elemental calcium; Aspara-Ca, Tanabe Seiyaku Co. Ltd., Osaka, Japan) per day was begun after the first angiotensin sensitivity test at 20 weeks of gestation until delivery. At each visit, information on the prescribed daily tablet treatment was collected by questioning the patients randomly. Patients not taking the prescribed calcium tablets accurately were excluded.

The angiotensin sensitivity test was carried out by a modification of the method of Gant et al.4 As a rule, it was performed in the morning in a quiet room. The patients were placed in a supine position and tilted about 15 degrees to avoid the hypotensive supine syndrome. Blood pressure was measured from the right arm by means of an automatic sphygmomanometer (BP-203, Nippon Kolin Co. Ltd., Tokyo, Japan) at 1minute intervals. After the diastolic blood pressure had become constant, an intravenous infusion containing I μg of angiotensin II amide (Hypertensin, Ciba-Geigy Ltd., Basel, Switzerland) per milliliter of 5% dextrose in water was given with an infusion pump (IVAC 530, Ivac Corporation, San Diego, California), and the in-

Table I. Nutritional assessment of daily diet in control and calcium-supplemented groups

	Control group	Calcium Erecp
Energy (kcal)	2007 ± 188	2285 ± ±€≤
Protein (gm)	89.5 ± 11.8	93.5 ± £5
Sodium (mg)	4926 ± 555	5319 ± °CE
Potassium (mg)	3218 ± 439	3517 ± 7生。
Calcium (mg)	876 ± 193	942 ± _4=*
Inorganic phosphorus (mg)	1378 ± 255	1419 ± 354

Except where indicated data were obtained for four patents in the control group and nine patients in the calcium-expolemented group. Data are expressed as the means ± SD. There were no significant differences between the control group and calcium-supplemented group.

fusion rate was increased by small increments every 5 minutes. The minimum amount of angiotensin I infused per kilogram of body weight per minute that caused a rise in diastolic blood pressure of 20 mm. Hg was defined as the effective pressor dose of angiotensin II. The angiotensin sensitivity test was carried but at 20, 26, 30, or 36 weeks of gestation. Among the 72 control pregnant women, 22 patients underwent the angiotensin sensitivity test at 20 weeks of gestation and one or more times at the other test periods. All of the calcium-supplemented pregnant women underwent serial testing.

Nutritional evaluations of the daily diet were made by a 24-hour recall method at 30 to 36 weeks of sestation. We selected randomly 10 patients in the calling supplemented group and 10 in the nonsupplemented group and asked them to note in detail the composition were as developed by Resources Council, span Science and Technology Agency.

Just before the angiotensin sensitivity test, blooc semples were collected by right antecubital venipurcure for determination of the serum ionized calcium: total calcium, total protein, albumin, sodium, potassium inorganic phosphorus, magnesium, creatinine, urie ecid, alkaline phosphatase, and immunoreactive parathy aid hormone. The serum ionized calcium concentration was measured with a calcium-specific electrode sandardized to pH 7.40 (Model ICAI, Radiometer Co. Ltd., Copenhagen, Denmark) under anerobic an I = xld conditions and analyzed in duplicate immediatels. The concentrations of serum total calcium, total profein, albumin, sodium, potassium, inorganic phospLczus, creatinine, uric acid, and alkaline phosphatase vere measured on an SMAC (Technicon Corporation, New York, New York). The serum magnesium level wa: neasured by the xylidyl blue method with the Magnes im B-Test Wako (Wako Pure Chemical Industries, L.d.,

Table II. Values for the effective pressor dose of angiotensin II during pregnancy

	Control group		Ca	alcium group
Gestational age (u4)	'n	EPD-AII	n	EPD-AII
. 2)`	22′	19.3 ± 2.1	22 .	18.1 ± 1.2
25	15	21.0 ± 2.2	20	$32.7 \pm 2.6*$
3)	19	18.6 ± 1.8	20	$41.1 \pm 3.4*$
35	10	15.4 ± 2.7	20	$25.9 \pm 2.9 \dagger$

EPD AII = effective pressor dose of angiotensin II, with values expressed as the means \pm SEM in ng/kg/min.

*Sigmificant difference compared to the control group: p < 0.001.

†Sigmificant difference compared to the control group: p < 0.005.

Osaka Japan). The serum immunoreactive parathyroid hormone (C-terminal 46-84 fragment) concentration was measured in duplicate by radioimmunoassay with the PTH Radioimmunossay Kit (Eiken Immunochemical Laboratory, Tokyo, Japan).

The data were analyzed for statistical significance by Student's t test and the χ^2 test.

Results

Effects on effective pressure dose of angiotensin II.

Angio-ensin sensitivity tests were carried out 128 times in the control group and 82 times in the calcium-supplemented group. Values for the effective pressure dose of angiotensin II during pregnancy are shown in Fig. 1. The effective pressure dose of angiotensin II value in the calcium-supplemented group was 18.1 ± 1.2 ng/kg/min (mean \pm SEM) before calcium admin.stration at 20 weeks of gestation, which was not significantly different from that in the control group. After calcium supplementation, the effective pressure dose of angiotensin II in the treated group was increased gradually until 30 weeks of gestation and was then decreased at 36 weeks of gestation. The levels were 32.2 ± 2.6 ng/kg/min (mean \pm SEM) at 26 weeks of gestation, 41.1 ± 3.4 ng/kg/min at 30 weeks, and 25.9 ± 2.9 ng/kg/min at 36 weeks. These values were significantly greater than those at 20 weeks of gestation (p < 0.001, p < 0.001, and p < 0.02, respectively). On the other hand, the effective pressure dose of angiotensia II in the control group continued at an almost constant level until 30 weeks of gestation and was then slightly decreased at 36 weeks of gestation. The levels in the control group were 17.7 ± 1.6 ng/kg/min (mean \pm SEM) at 26 weeks of gestation, 17.6 \pm 1.2 ng/ kg/min at 30 weeks, and 15.0 ± 1.6 ng/kg/min at 36 weeks. These values were significantly smaller than those in the calcium-supplemented group (p < 0.001, p < 0.001, and p < 0.005, respectively).

^{*}Seven cases only.

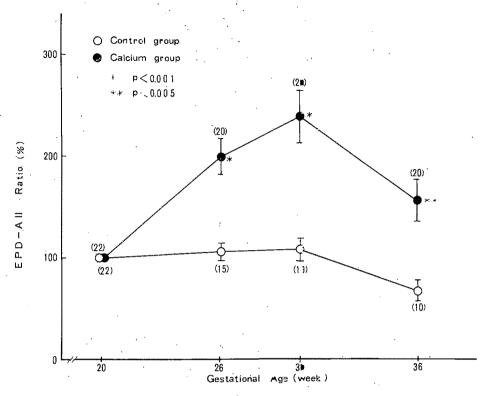


Fig. 2. Effective pressor dose of angiotensin II (EPD-4II) ratios when the values at 20 weeks of gestation before calcium supplementation were taken as 100% in the control group (0) and the calcium-supplemented group (•). Values are expressed as the mean ± SEM. Significant differences compared to the control group: * = p < 0.001; ** = p < 0.005.

Nutritional assessments of the daily diet in the control group and the calcium-supplemented group are shown in Table I. Only four of the 10 subjects in the control group and nine of the 10 patients in the treated group gave reliable answers, because the 24-hour recall method is rather complicated. The data for the diet of the calcium-supplemented group were not significantly different from those in the control group.

In order to examine the changes in vascular sensitivity to angiotensin II in the individual patients, we calculated the effective pressor dose of angiotensin II ratio in each patient, with the value for the initial angiotensin sensitivity test at 20 weeks of gestation taken as 100%. Of the 72 nonsupplemented pregnant women, 22 patients underwent the angiotensin sensitivity test at 20 weeks of gestation and one or more times at the other test periods. The values for the effective pressor dose of angiotensin II in these 22 patients were not significantly different from those in the 72 nonsupplemented pregnant women. The effective pressor dose of angiotensin II values in the 22 control pregnant women and 22 calcium-supplemented pregnant women are listed in Table II, and the effective pressor dose of angiotensin II ratios are shown in Fig. 2. The patterns of change in effective pressor dose of. angiotensin II ratios were similar to the changes in the values. The effective pressor dose of angiotensin II ratios in the control group were significantly smaller than those in the calcium-supplemented group.

Effect on blood chemical parameters. The blood chemical findings in the calcium-supplemented pregnant women were compared with those of eight of the 75 nontreated pregnant women from whom blood samples could be taken serially. The blood chemical parameters in the calcium-supplemented women and nonsupplemented pregnant women are summarized in Table III. The values for each parameter in the two groups were not significantly different from each other.

Clinical outcome and calcium supplementation. Of the 72 control patients, 66 were followed up until term. All patients in the calcium-supplemented group were fo lowed up until term. The outcomes of the pregnancies in these 66 control patients and 22 calcium-supplemented patients are shown in Table IV. No side effects of the calcium administration were observed. The dosage of calcium did not reduce the blood pressure, but there was a slight increase up to the late third trimester. The systolic baseline blood pressure levels at the time of the angiotensin sensitivity test were 97.5 ± 7.0 mm Hg (mean \pm SD) at 20 weeks of ges-

Table III. Blood chemical data during pregnancy

•	Gestational age							
. Parameter	20 wk	n	26 wk	n	30, wk	n	36 wk	n
Ionized calcium (1.19-1.27 mmol/L*)								
Normal control	1.17 ± 0.01	3	$^{\circ}1.20 \pm 0.02$	4	1.20 ± 0.01	4	1.21 ± 0.05	4
Calcium supplementation	1.21 ± 0.05	13	1.21 ± 0.03	12	1.18 ± 0.03	5	1.20 ± 0.03	5
Total calcium (8.7-10.3 mg/100 ml)								
Normal control	8.84 ± 0.36	5	8.97 ± 0.56	6	8.79 ± 0.34	7	8.83 ± 0.20	7
Calcium supplementation	9.25 ± 0.43	22	8.94 ± 0.31	21	8.80 ± 0.32	20	8.89 ± 0.46	21
Albumin (4.1-5.1 gm/100 ml)	•		2 × 1	٠.				
Normal control	3.72 ± 0.12	6	3.65 ± 0.12	. 6	3.63 ± 0.15	· 7	3.70 ± 0.13	7
Calcium supplementation	3.74 ± 0.25	22	3.59 ± 0.21	21	3.53 ± 0.20	20	3.48 ± 0.28	21
Magnesium (1.5-2.5 mg/100 ml)			*				•	
Normal control	1.83 ± 0.07	6	1.93 ± 0.16	5	1.93 ± 0.10	3	1.99 ± 0.07	7
Calcium supplementation	1.90 ± 0.09	22	1.95 ± 0.11	21	1.90 ± 0.10	13	2.00 ± 0.12	19
Immunoreactive PTH (0.5 ng Eq/ml)	,		•		•			
Normal control	0.40 ± 0.11	6	0.30 ± 0.08	6	0.35 ± 0.11	7	0.29 ± 0.08	7
Calcium supplementation	0.32 ± 0.11	22	0.33 ± 0.11	21	0.40 ± 0.12	20	0.37 ± 0.12	20

Data were obtained from eight patients in the normal control group and 22 patients in the calcium-supplemented group. Data are expressed as the means \pm SD. None of the paired $4 \pm a$ were significantly different.

Table IV. Clinical outcomes of pregnancy in 66 cntrol pregnant women followed up to term and in 22 calcium-supplemented pregnant women

	Gestational age (wk)	Birth weight (gm)	Apgar score (1 min)	Incidence of pregnancy-induced hypertension*
Control group	39.0 ± 2.1	2971 ± 584	8.3 ± 1.5	21.2
Calcium group	39.5 ± 1.2	3169 ± 303	8.6 ± 0.7	4.5

Data are expressed as the means ± SD. There were 10 significant differences between the control group and calcium-supplemented group.

tation, 99.2 ± 7.7 mm Hg at the twenty-sixth v c≥k, 99.9 ± 8.2 mm Hg at the thirtieth week, and 105.6 ± 9.5 mm Hg at the thirty-sixth week; baseine diastolic blood pressure values were $46.8 \pm \tilde{z}.3$, 51.0 ± 6.7 , 53.9 ± 6.8 , and 56.3 ± 8.7 mm Hg. respectively. These blood pressure levels for the calcumsupplemented group were not significantly different from those in the control group. There was only the case of pregnancy-induced hypertension in the calcium-supplemented patients. This patient had zen normotensive preceding pregnancy, and hypertersion occurred at 36 weeks of gestation and persisted during the 2 months post partum that we were able to fclow up. She was delivered of a 3110 gm infant at 39 weeks of gestation. The incidence of pregnancy-inducec hypertension in the calcium-supplemented patients was 4.5%. On the other hand, 14 of the 66 control patents developed pregnancy-induced hypertension. Thus the incidence in the control group was 21.2%, which although larger than that in the calcium-supplemented group, did not represent a statistically significant zifference between the two groups ($\chi^2 = 3.24$, 0.05).

Comment

The effects of normal pregnancy on calcium metabolism have been well investigated and it has been shown that pregnancy is generally associated with physiologic hyperparathyroidism.8 Various studies have assessed the relationship between calcium and blood pressure. Oral calcium intake or administration has been shown to reduce the blood pressure in nonpregnant and pregnant women.7.9 Belizán et al.7 demonstrated that patients receiving 2 gm/day of calcium had lower blood pressure values throughout the third trimester than patients receiving 1 gm/day of calcium and suggested that a fair correlation existed between dosage of calcium and blood pressure. In the present study, a lower dose of supplemented calcium (156 mg/day of elemental calcium) produced a significant reduction in vascular sensitivity to angiotensin II, which is said to precede an alteration in blood pressure without decreasing the

^{*}Range in normal healthy adult.

^{*} χ^2 test, 0.05 .

blood pressure. For ethical reasons, we were unable to administer placebo tablets to control pregnant women, so we could not exclude a possible effect of L-aspartate. However, there are no reports to suggest that L-aspartate affects the blood pressure or the vascular sensitivity to pressor agents.

There is wide agreement that loss of vascular refractoriness to angiotensin II plays one of the most important roles in the pathogenesis of pregnancy-induced hypertension. It has been considered, therefore, that restoration of angiotensin II refractoriness may by some means be able to prevent the onset of pregnancyinduced hypertension, and discoveries along these lines are expected. Several reports have indicated reduction of vascular sensitivity to angiotensin II in pregnant women, Everett et al.10,11 by the oral administration of theophylline and by infused 5α-dihydroprogesterone infusion and Kaulhausen et al.12 by the oral administration of L-dopa. However, these drugs seem to be dubious as regards maternal and fetal side effects with long-term use for preventing the onset of pregnancyinduced hypertension. In the calcium-supplemented pregnant women in the present study, the outcomes of pregnancy were almost totally successful and side effects were not observed. Calcium administration is considered to exert little effect on the fetus, since it occurs in the body sufficiently and to spare.

From the viewpoint of prevention, it does not seem to be necessary to reduce the blood pressure. Therefore, ingesting adequate calcium may valuable for preventing the onset of pregnancy-induced hypertension. We could not readily assess the effect of calcium supplementation on the blood pressure in the present study group, since both the control group and the calcium-supplemented group included some patients with pregnancy-induced hypertension and the incidences in the two groups were different. However, the blood pressure in the calcium-supplemented patients did not decrease from the level at 20 weeks of gestation, so that the dosage of supplemental calcium did not appear to reduce the blood pressure. Further, it led to a lower incidence of pregnancy-induced hypertension in the calcium-supplemented group (4.5%) than in the control group (21.2%), although the level of statistical significance between the incidences was borderline. The incidence in the control group appears to be high and may have reflected the fact that there were many patients with some risk factors of pregnancy-induced hypertension, such as a family history of hypertension or diabetes, a past history of renal disease, hypertension, preeclampsia, or endocrinologic disorders, and elderly primiparous patients. Nevertheless, similar to the control group, many patients with high-risk factors were in the calcium-supplemented group. To assess the true preventive value of calcium supplementation for pregnancy-induced hypertension, studies with larger numbers of subjects are in progress at our laboratory.

There is no clear explanation of the mechanisms for such effects of calcium on the vascular system and blood pressure, although calcium supplementation may affect the calcium homeostatic mechanisms. Parathyroid hormone, calcitonin, and 1a,25-dihydroxycholecalciferol are known to be major calcium-regulating hormones. Belizán et al.7 suggested that one possible explanation of the effect of calcium on blood pressure involved parathyroid hormone, since a partial coefficient correlation between parathyroid hormone levels and diastolic blood pressure was observed. In the present study, the serum immunoreactive parathyroid hormone levels were found to be almost constant during pregnancy. In the literature, the relationship of parathyroid hormone to the vascular system remains conflicting. 13.14 Published data concerning the maternal calcitchin levels in pregnancy are sparse.8, 15 There have also been few reports evaluating the relationship of 1α,25-dihydroxycholecalciferol to the vascular system. More basic research is required to assess the possible role of the major calcium-regulating hormones.

There is one other interesting observation which has a bearing on the possible explanation of the present results Bohr¹⁶ found that a high calcium concentration depressed the vascular smooth muscle response, probably by binding to the muscle cell membrane, thereby stabilizing it, reducing its excitability, and inhibiting its contraction. In the case of a slow and low-grade increase in the extracellular calcium concentration, such as in dietary calcium supplementation, calcium homeostasis may be maintained through binding to the cellular membrane before the major calcium-regulating hormones come into operation.

In conclusion, we found calcium supplementation to be an important factor which can reduce the vascular sensitivity to infused angiotensin II. Although the mechanisms of this effect of calcium supplementation remair unclear, it is suggested that calcium supplementation or an increase in dietary calcium intake can help to prevent the onset of pregnancy-induced hypertension.

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The relationship between feral activity and behavioral states and fetal breathing movements in normal and growth-retarded fetuses

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The incidence of fetal breathing was studied during the course of behavioral state observations on 28 low-risk fetuses between 32 and 40 weeks' gestational age and on 12 growth-retarded fetuses between 36 and 40 weeks. Real-time ultrasound scanners were used to detect fetal eye, body, and breathing movements, and the fetal heart rate was recorded continuously. The mean duration of the observation sessions was 110 minutes. The mean incidence of fetal breathing was greater during periods of fetal activity (body and eye movements present, greater heart rate variability) than during quiescence (body and eye movements absent, narrowed heart rate variability) a ell gestational ages studied in both low-risk and growth-retarded fetuses. During periods when one of the state variables (body movements, eye movements, heart rate pattern) was in its active condition while the other two were quiet, or the reverse, the incidence of fetal breathing was intermediate between those found when all three state variables were in agreement. After behavioral states had developed, at and 40 weeks, the mean incidence of fetal breathing in the low-risk fetuses was greater during active states than during the quiet state. There was no apparent increase in the degree of linkage between fetal preathing and other expressions of fetal activity after the emergence of behavioral states. (AM J Obster Camecol 1985;153:582-8.)

Key words: Fetal behavior, fetal breathing, fetal movements, fetal heart rate, fetal growth retardation, real-time ultrasound

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Soon after the rediscovery of fetal breathing movements, it became apparent that these movements were highly related to some other expressions of central nervous system activity. In fetal lambs after differentiation of electrocorticographic activity patterns, continuous fetal breathing is limited almost exclusively to periods of low-voltage, high-frequency electrocortical activity, when eye movements are also present. In the human

State criteria	State 1F	State 2F	State 3F	State 4F
Body movements	Incidental	Periodic	Absent	Continuous
Eye movements	Absent	Present	Fresent	Present
Heart rate pattern	Α	В	С	D

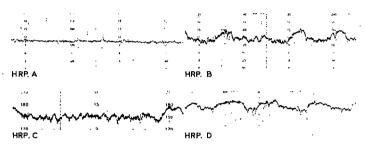


Fig. 1. Summary of the criteria used for defining the behavioral states of the human fetus.

fetus Timor-Tritsch et al.2 observed that fetal breathing movements (detected by external tocodynamometry) did occur during periods of somatic quiescence but that their incidence then was less than when the fetus was active. This finding was confirmed by Junge and Walter,3 who used real-time ultrasound scanning to detect fetal breathing movements. Since in both of these studies fetal sleep state was defined on the basis of fetal body movements and heart rate pattern, it is possible that the differences found in the incidence of fetal breathing may have been related more to a rest-activity cycle than to sleep or behavioral states.

The human fetus shows periods of increased and decreased somatic motility from an early age.4.5 Behavioral states, however, are "distinct conditions, each having its specific properties and reflecting a particular mode of nervous function."6 The state concept implies linkage of several physiologic variables. Consequently, behavioral state cannot be assigned solely on the basis of variations in somatic motility and changes in the fetal heart rate, for heart rate is unquestionably affected by somatic activity and changes in heart rate may simply be the result of quantitative differences in movement. Breathing patterns together with somatic motility and opening and closing of the eyes are the variables used to define behavioral state in the newborn infant.6 Since breathing is not continuously present in utero, its rhythm cannot be used as a criterion for defining behavioral states of the fetus.

With the demonstration that fetal eye movements can be detected by means of real-time ultrasound scanning, however, it became possible to investigate the presence of behavioral states prior to birth by substituting eye movements, which are highly state-dependent,6 for opening and closing of the eyes. Somatic motility and fetal heart rate patterns, particularly the variability of the baseline heart rate, were used as the other state variables. In near-term fetuses, four states could be identified that are analogous to states one through four of the newborn infant. Since the criteria used to define these states differed from those used in the infant, the suff x F was added to the state designation to signify "fetal." The definitions of these states are summarized in Fig. 1. It was found that behavioral states develop at 35 to 38 weeks' gestational age in the fetuses of lowrisk multiparous women8 and about 2 weeks later in the fetuses of low-risk nulliparous women.9 Before the emergence of states, the state variables do occur in the defined combinations, but the relatively short durations and instability of these associations, and the lack of synchrony of change of all three variables at transitions from one defined combination to another, indicate that the linkage between the variables, if present, is incomplete. Detailed discussions of the emergence of behavicral states are presented in the original publications.3,9

The purposes of this study were to investigate the influence of behavioral state on the incidence of fetal breathing in the term fetus and to observe the effect of changing levels of fetal activity on breathing in the preterm fetus before states have emerged. Also, the findings in low-risk fetuses were compared with those obtained by means of the same techniques in growthretarded fetuses.

Subjects and methods

Behavioral state observations were carried out serially at 2-week intervals from 32 weeks' gestation until term or the fetuses of 14 low-risk multiparous women and 14 low-risk nulliparous women. Criteria for inclusion in the low-risk group were singleton pregnancy with reliable dates, no maternal medical illness or antepartum obstetric complication, and no medication (other than iron supplements). For the parous women,

Table I. Numbers and mean durations of recordings at each gestational age from fetuses of low-risk gravid women

			Gestational age (wk)		
	32	34	36	38	40
No. of subjects					
Nulliparous	6	12	13	13	7
Multiparous	13	· 14	· 14	14	9
Recording time (min)				·	
Mean	94	104	. 114	115	117
Range	60-129	60-124	69-135	76-145	60-138

Table II. Numbers and mean durations of recordings at each gestational age from growth-retarded fetuses

!	Ges	tational age	(wk)
	, 36	38	40
No. of subjects Recording time (min)	5	7	Ξ
Mean Range	121 16-128	114 88-120	1년 94-126

the prior pregnancy or pregnancies must have seen uncomplicated and ended in the birth of a normal infant at term. Ten of the 28 mothers smoked. seen fewer than 10 cigarettes per day and three between 10 and 15 cigarettes per day. For various reason some fetuses could not be studied at every gestational age interval. The numbers of fetuses studied at each gestational period and recording times are summanized in Table I.

Behavioral state observations were also mace prospectively on 12 additional fetuses, suspected antepartum of being growth-retarded and having e-emalal birth weights below the tenth percentile for the Durch population.10 The criteria for inclusion in this grap, besides clinical and ultrasound findings indicating fetal growth retardation, were similar to those for the lowrisk group except that women with mild nonprotein ric hypertension were not excluded. Cases in which congenital anomalies or transplacental infection was suspected as the cause of the growth retardation were excluded. Nine of the 12 mothers smoked, in eac instance fewer than 10 cigarettes per day. Seven ercwthretarded fetuses were studied only once; two_ take; and three, three times. The numbers of fetuses #udled at each gestational period and the recording times for this group are given in Table II.

All observations were carried out between 700 PM and 9:30 PM starting approximately 1 hour after the mother had eaten her normal evening meal, to minimize variations due to maternal food intake and directional rhythms. The methods used in the fetal behavioral state observations have been described previously in de-

tail^{8,9} and will only be summarized here. Fetal eye, face, body, and breathing movements were detected by means of two real-time ultrasound scanners (Toshiba SAL 20A, 2.4 mHz; Philips Sono Diagnost LA 1012, 3.0 mHz), positioned respectively to obtain an oblique parasagittal section through the fetal face and a transverse section at the level of the upper abdomen. The fetal heart rate was recorded continuously by means of a clinical fetal monitor (Corometrics 112 or Toitu MT 820). The images of the fetal face and eye were stored on videotape for later analysis. Fetal body and breathing movements were recorded verbally on the audio channel of the video recorder.

The videotape was replayed following the observation session. Fetal eye and body movements and the beginning and end of episodes of fetal breathing were marked on the uterine contraction channel of the fetal monitor with a five-key event marker. The resulting actogram was combined with the heart rate recording by means of synchronizing signals placed regularly during the observation session.

Each of the three fetal behavioral state variables (body movements, eye movements, and fetal heart rate pattern) and fetal breathing were analyzed separately for condition (parameters for body, eye, and breathing movements, presence or absence; for heart rate, patterns A through D, Fig. 1) with the use of a 3-minute moving window advanced in half-minute steps. The result of this procedure was a profile showing the changes of each variable between its possible conditions (parameters). The three behavioral state variables were then analyzed simultaneously to identify epochs in which the parameters of all three were present in the previously defined combinations (Fig. 1).^{8, 9}

Since the state variables show periodic changes in their conditions, specific combinations of parameters may occur by chance because of overlap or as a result of linkage of the variables. To allow for this, periods during which the parameters of the state variables were present in the combinations defined in Fig. 1 were called periods of coincidence *1F* to *4F*, respectively. Epochs during which the parameters of the state variables did not fit one of the defined combinations were

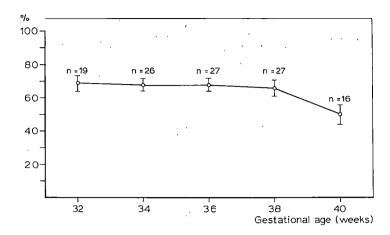


Fig. 2. Incidence of fetal breathing movements (means \pm SEM) during the total recording time in the low-risk fetuses.

called periods of no coincidence. To accept the presence of behavioral states, two additional criteria had to be met. The first of these was duration of stable association: The defined combinations of the state variables had to persist for a minimum of 3 minutes without interruption. The second and more important criterion was simultaneity of change. Transitions from one of the specified combinations to another had to be completed within a maximum of 3 minutes, without a longer intervening period of discordant association (no coincidence). Only when these two criteria were met during the majority of a recording did we deem the linkage between variables sufficient to conclude that behavioral states were present.8,9

Results are presented as means and standard errors. The significance of differences was assessed by means of the Wilcoxon matched pairs-signed ranks test of comparisons within groups, and the Mann-Whitney U test between groups. The significance levels are for twosided tests. Because multiple comparisons were carried out (that is, for 32, 34, 36, 38, and 40 weeks), a significance level of 0.01 was used.

Results

Low-risk subjects. Pregnancy was uncomplicated in 26 of 28 women classified prospectively into this group. Two developed late nonproteinuric hypertension at 36 and 41 weeks, with highest diastolic pressures of 95 and 100 mm Hg, respectively. All were delivered vaginally. All fetuses in this group had birth weights above the tenth percentile for gestational age and Apgar scores of 9 or 10 at 5 minutes. In only one case was the pH of umbilical cord blood at delivery below the tenth percentile for low-risk patients at this hospital.11

There were no significant differences in the incidences of fetal breathing between the fetuses of nulliparous and multiparous women at the range of gestational ages studied. Therefore, these two groups were combined in the further analyses. Fig. 2 shows the mean incidence of fetal breathing obtained with the 3-minute window technique, from 32 to 40 weeks' gestational age. The decrease between 38 and 40 weeks was not significant in the 16 fetuses recorded at both times. There was also no significant difference in the incidence of fetal breathing between the fetuses of mothers who smoked and those who did not.

The incidence of fetal breathing during epochs of coincidence IF (quiescence) and 2F (periodic somatic activity, eye movements present), and of no coincidence is shown in Fig. 3. Fetal breathing was present during a smaller proportion of the time in coincidence 1F than in coincidence 2F at all ages studied (p < 0.01). Fetal breathing during epochs of no coincidence was intermediate between these two, being significantly less than during coincidence 2F at all ages and greater than during coincidence 1F at 34, 36, and 38 weeks (p < 0.01). The incidence of fetal breathing during coincidence 4F (continual vigorcus body movements) was similar to that observed during coincidence 2F.

Behavioral states were present in 19 of 27 fetuses studied at 38 weeks and in 15 of 16 at 40 weeks. The incidence of fetal breathing during state 1F and 2F epochs in these fetuses is shown in Fig. 4. Fetal breathing was present significantly more of the time during state 2F than during state 1F at both ages (p = 0.001). No state could be identified during an average of 12% of the recording time in these fetuses at 38 weeks and 4% of the time at 40 weeks. During these epochs the incidence of fetal breathing was intermediate between those in states 1F and 2F, but the only significant difference was with the incidence of breathing during state 1F at 38 weeks (p = 0.01). The incidence of fetal breathing during state 4F epochs was similar to that during state 2F.

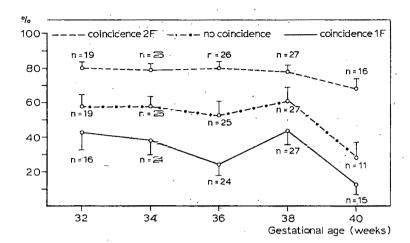


Fig. 3. Incidence of fetal breathing movements (means \pm SEM) in the low-risk fetuses during periods of coincidence 1F, coincidence 2F, and $\neg \bullet$ coincidence.

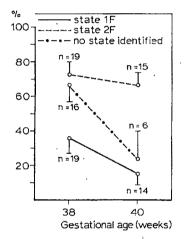


Fig. 4. Incidence of fetal breathing movements (m- and \pm SEM) during behavioral states IF and 2F and during periods when no state could be identified in the low-risk fetuses at 38 and 40 weeks' gestational age.

Eight fetuses did not have states present at 38 weeks. In these fetuses, the mean incidence of breathing furing coincidence 2F ($78\% \pm 8\%$) was similar to that furing state 2F in the fetuses with states present ($78\% \pm 7\%$). The incidence of breathing during coincidence 1F in the fetuses that had not developed states was somewhat higher than during state 1F in the fetuses with states ($53\% \pm 18\%$ versus $36\% \pm 9\%$), but this limerence was not significant.

Since the fetal heart rate pattern is often the only clinical documentation of the level of fetal activity, we analyzed the incidence of fetal breathing during fetal heart rate patterns A and B (Fig. 5). This was fen ficantly greater during periods when fetal heart rate pattern B (wider variability band width, accelerations) was present than during fetal heart rate pattern A (range) wariability band width, no accelerations) at all eges studied (p = 0.01).

Growth-retarded fetuses. None of these Eruses

showed any evidence of fetal distress at the time of the observation sessions, but two subsequently developed decelerations of the fetal heart rate and were delivered by cesarean section in the thirty-sixth and thirty-ninth weeks of pregnancy. The rest were delivered vaginally between 38 and 42 weeks. The Apgar scores at 5 minutes were 9 or 10 in all cases, and no fetus was severely acidotic at birth (lowest umbilical arterial pH, 7.18). Three birth weights were below the 2.3 percentile for gestational age, five between the 2.3 and fifth percentiles, and four between the fifth and tenth percentiles.

Fig. 6 shows the mean incidence of fetal breathing at 36, 38, and 40 weeks in the growth-retarded fetuses together with the corresponding values for the low-risk fetuses. Although the incidence of breathing was lower at each age in the growth-retarded fetuses, none of the differences was significant (also not at the 0.05 level).

Fig. 7 shows the incidence of fetal breathing during periods of coinc dence IF and 2F and of no coincidence in the growth-retarded fetuses, and Fig. 8 gives this incidence for heart rate patterns A and B. The pattern of occurrence of fetal breathing in both instances was similar to that observed in low-risk fetuses, but because of the smaller rumbers, the significance level was not reached. The p values ranged between 0.03 and 0.06. Only two growth-retarded fetuses had behavioral states at 38 weeks, and three, at 40 weeks. Each of these fetuses showed a lower incidence of fetal breathing during state IF epochs than during state 2F periods.

Comment

The incidence of fetal breathing reported here is much higher than that cited in most other reports. The difference is due to our use of a 3-minute moving window to assess the presence of fetal breathing as well as body and eye movements and heart rate patterns. This is a smoothing procedure that emphasizes the stable properties of the variables and eliminates short-term

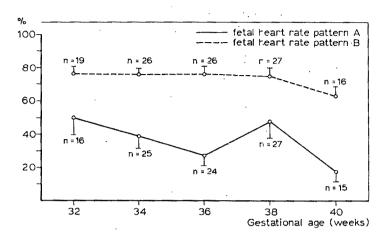


Fig. 5. Incidence of fetal breathing movements (means \pm SEM) in the low-risk fetuses during fetal heart rate patterns A and B.

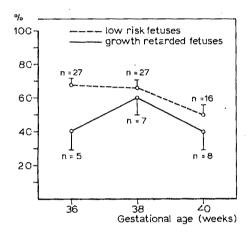


Fig. 6. Incidence of fetal breathing movements (means \pm SEM) in the low-risk and growth-retarded fetuses between 36 and 40 weeks' gestational age.

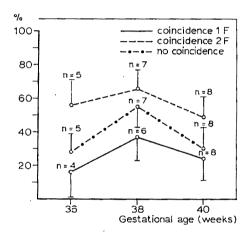


Fig. 7. Incidence of fetal breathing movements (means \pm SEM) in the growth-retarded fetuses during periods of coincidence 1F, coincidence 2F, and no coincidence.

fluctuations. For the study of behavioral states, these stable properties are more important than the transient disturbances.⁶ The result of this method of analysis is that a period of apnea must exceed 3 minutes before breathing is said to be absent, whereas most other investigators have used much shorter criteria for apnea, usually 6 seconds. While a short burst of fetal breathing during a longer period of apnea will not be recorded with the use of a relatively broad window, this situation occurs much less frequently than interruptions of fetal breathing during periods when it is predominantly present.

The incidence of fetal breathing in the growth-retarded fetuses in this study was not significantly different from that in the low-risk group. This is at variance with the results in the literature. 12, 13 The explanation may lie in differences in the populations studied, since our selection criteria excluded growth retardation secondary to severe maternal hypertension and vascular disease, and other patients with early, severe growth retardation were usually transferred to the uni-

versity hospital only after fetal distress requiring termination of the pregnancy had appeared. Most of the growth-retarded fetuses in the study group had the typical wasted appearance of disproportionate growth retardation at birth, but none was genuinely distressed and 10 of the 12 pregnancies in this group were allowed to proceed to spontaneous termination. Another possibility is that use of the 3-minute window may have overestimated the incidence of breathing by the growth-retarded fetuses to a greater extent than in the low-risk group, for Trudinger et al.14 reported that some growth-retarded infants showed short breathing episodes with longer apneic intervals than those of normal fetuses. Inspection of the recordings from our growth-retarded fetuses did not give any indication of differences in breathing pattern between them and the low-risk group, although we did not specifically measure the durations of the apneic periods.

Fetal breathing was present during a greater proportion of the time when fetuses were active than when they were quiescent. This was found at all gestational

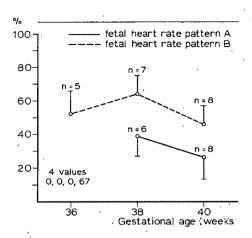


Fig. 8. Incidence of fetal breathing movements (means \pm SEM) in the growth-retarded fetuses during fetal heart rate patterns A and B.

ages studied in both the low-risk and growth-related fetuses and with all three indicators of fetal a tivity: coincidence of behavioral state variables, behavioral states, and fetal heart rate patterns. Our results are true in agreement with the observations of Timor-Trusch et al.² and Junge and Walter³ in term fetuses, where activity state was assessed only from somatic modity and heart rate. Also, in the study of Timor-Trusch et al., the incidence of fetal breatning during finitermediate sleep"—epochs that did not fulfill the criteria for either active or quiet sleep—was intermediate between the incidence in active and quiet sleep, as was found for epochs of no coincidence in this study.

Our observations indicate that there is a linkage between fetal breathing movements and other expressions of fetal activity and quiescence. When fetz body and eye movements are "on" and fetal heart rate rariability is increased (coincidence 2F and coincidence F), fetal breathing is also "on" a greater proportion of the time than it is "off." The reverse applies to periods when body and eye movements are "off" and Feart rate is stable. During periods of discordant association (no coincidence), when some variables are "or" and others "off," the incidence of breathing was in-ermediate between the levels found in concordant quiescence (coincidence 1F) and activity (coincidenc∈2F or 4F). This linkage is present before behavioral sates develop, and in contrast to the linkage among the behavioral state variables themselves, it does not seem to increase after the states develop; the range of incidences of fetal breathing during state 1F in The 19

fetuses with states at 38 weeks was just as wide as that during coincidence IF in the eight fetuses without states at this age. On the other hand, differences are present in the rhythmicity of fetal breathing between states IF and 2F, ¹⁵ demonstrating that breathing is linked to behavioral state before birth, as it is afterward.

Our findings also indicate that in studies of human fetal breathing, the effects of the condition or procedure being investigated on other aspects of fetal activity must be taken into account, for it is possible that an observed change in fetal breathing may be secondary to an effect on fetal activity or behavioral state.

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Inhibition of arachidonic acid metabolism by antipyrine and 4-aminoantipyrine

D. Cohen, J. Corbin, J. P. Figueroa, M.D., P. W. Nathanielsz, M.D., Ph.D., and M. D. Mitchell, D.Phil.

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Antipyrine and 4-aminoantipyrine are used to determine uterine and umbilical b ood flows. When administered in vivo, these compounds produce a decrease in uterine contracture activity and maternal uterine vein 13,14-dihydro-15-keto-prostaglandin F_{2a} concentrations. In this paper we report that they also inhibit in vitro activity of prostaglandin synthase in bovine seminal microsomes. (AM J OBSTET GYNECOL 1985;153:589-90.)

Key words: Antipyrine, aminoantipyrine, prostaglandin synthase

Antipyrine and 4-aminoantipyrine are used commonly in the determination of rates of blood flow.1.2 Recently we have shown that administration of these compounds to sheep during late pregnancy results in a significant diminution in uterine contracture activity.3 Concomitantly there was a reduction in the plasma concentration of 13,14-dihydro-15-keto-prostaglandin F₂₀. It is our belief that antipyrine and 4-aminoantipyrine inhibit uterine contracture activity by way of inhibition of prostaglandin biosynthesis. To substantiate this hypothesis we have investigated the effects of antipyrine and 4-aminoantipyrine on prostaglandin synthase, that is, a microsome-enriched preparation of bovine seminal vesicles. Furthermore, we have determined the actions of antipyrine and 4-aminoantipyrine on arachidonate lipoxygenase activity, another major pathway of arachidonic acid metabolism.

Material and methods

Antipyrine was obtained from three different sources: Sigma Chemical Company, Pfaltz and Bauer, Inc., and Matheson, Coleman, and Bell. 4-Aminoantipyrine was obtained from Sigma Chemical Company. Actions on prostaglandin synthase and arachidonate lipoxygenase were determined by methods that have been fully described and validated elsewhere.^{4, 5} The total incubation volume in each method was 1 ml.

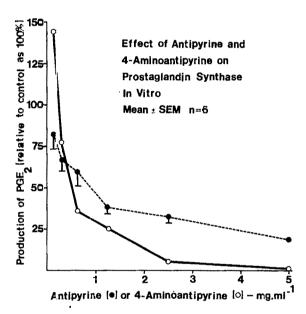


Fig. 1. The effects of antipyrine and 4-aminoantipyrine on the biosynthesis of prostaglandin E_2 by a microsome-enriched preparation of bovine seminal vesicles. Values for antipyrine are the means of duplicate determinations \pm SEM on each of three different batches of the compound. Values for 4-aminoantipyrine are the means of duplicate determinations.

Result

Both antipyrine and 4-aminoantipyrine caused a concentration-dependent inhibition of prostaglandin synthase activity (Fig. 1). With use of a one-way analysis of variance, the inhibition with antipyrine was significant (> < 0.01). No statistical analysis was performed for the 4-aminoantipyrine data, since measurements were only performed in duplicate. In the case of 4-aminoantipyrine there was a tendency for increased synthase activity at very low concentrations; this stimulating effect was not evident with antipyrine. There was no similar clear-cut effect of these compounds on arachidonate lipoxygenase activity over the same range

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Reprint requests: Peter W. Nathanielsz, M.D., Ph.D., Reproductive Studies, College of Veterinary Medicine, Cornell University, Ithaca, NY 14853. of concentrations (0.15-5 mg·ml⁻¹). Neverthel s. at high concentrations of 4-aminoantipyrine a small degree of inhibition of lipoxygenase activity was observed (data not shown).

Comment

These results are consistent with the view that a tipyrine and 4-aminoantipyrine can attenuate uprine contracture activity by an action on prostaglandic synthesis. Moreover, the greater potency of 4-amine antipyrine with respect to inhibition of prostaglandir synthesis is consistent with our observation on their realive potencies in vivo with respect to inhibition of uterine activity. The results of our studies concerning anacindonate lipoxygenase activity are inconclusive but siggest that the major actions of antipyrine and 4-a-rinoantipyrine on arachidonate activity are by way of actions on the prostaglandin biosynthetic pathway Tre inhibitory effects of antipyrine and 4-aminoantipyrine may result from alteration of the balance of products of the cycloxygenase and lipoxygenase pathways. This hypothesis is currently being tested in vivo. In summary, these results confirm our previous evidence that

prostaglandins play a role in the control of contractures. They also suggest caution in the use of these two compounds, especially 4-aminoantipyrine, to measure blood flows in the pregnant animal.

We thank Dr. Paul C. MacDonald for the use of laboratory facilities.

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CORRESPONDENCE

Relief of pain in first-trimester abortion

To the Editors:

I would like to ask Dr. Suprapto and Ms. Reed about their article, "Naproxen sodium for pain relief in first-trimester abortion" (AM J OBSTET GYNECOL 1984; 150:1000-1).

They state they used a 1% lidocaine solution for paracervical block. However, they did not state the amount of lidocaine or the amount of time before the procedure that the lidocaine was administered. Some physicians have found that although lidocaine has a fast onset of action, its duration of effect is only moderate. Thus the latency time would significantly affect the amount of pain perceived.

In the article they also did not mention any use of laminaria as an adjunct to cervical dilatation. In termination of pregnancies, patients with very stenotic cervixes, sharply anteverted or sharply retroverted uteruses, or previous surgical procedures are helped considerably by the use of laminaria tents with good success rate for a more gentle cervical dilatation and decreased pain during the procedure. I wondered if they assessed any of their patients for the laminaria and, if so, were these patients removed from the study group.

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Use of intravenous terbutaline to facilitate uterine repositioning

To the Editors:

We read with interest the report by Kovacs and DeVore (AM J OBSTET GYNECOL 1984;150:784) of the use of intravenous terbutaline to facilitate uterine repositioning in two patients with postpartum uterine inversions. The authors suggested that in the patient with uterine inversion but without hemorrhage or signs of shock, terbutaline represents an alternative to the use of halothane anesthesia for uterine replacement.

We concur, but since shock and hemorrhage frequently occur in this obstetric emergency, we have favored the use of magnesium sulfate. This tocolytic agent is without the undesirable cardiovascular effects of terbutaline. One case in which magnesium sulfate was successfully used for uterine repositioning has been previously reported. Four patients with acute postpartum uterine inversion in whom replacement could not be accomplished without tocolysis have been treated here. Terbutaline was used in two cases, and magnesium sul-

fate, 2 to 4 gm intravenously over 3 to 5 minutes, was used in two others. In each case following tocolytic treatment, the uterus was repositioned without recourse to the use of halothane anesthesia (Catanzarite VA, et al. New approaches to the management of acute puerperal uterine inversion. Unpublished observations).

Following uterine replacement, hemorrhage may continue until uterine tone is reestablished, and reinversion is not uncommon. In one of the cases reported by Kovacs and DeVore, oxytocin administration did not restore uterine tone for 10 minutes, and in the other, vaginal packing and oxytocin were used to maintain position. In one of our cases, oxytocin was not adequate to maintain position, and abdominal uterine suspension was required.

Prostaglandin $F_{2\alpha}$ and a synthetic analogue, 15-(s)-15-M-prostaglandin- $F_{2\alpha}$ (Prostin-15M), are uterotonic agents more potent than oxytocin, at least in the treatment of postpartum uterine atony. Theoretical side effects are wheezing in asthmatic patients and increased blood pressure. Neither of these has been a significant problem in reported series. We are aware of one prior case in which Prostin-15M was successful in treating recurrent inversion following uterine replacement. In four patients so treated here, including three who had received tocolytic agents (one of whom had recurrent inversion), intramyometrial injection of Prostin-15M in doses of 125 to 250 μ g resulted in prompt uterine contraction, control of bleeding, and maintenance of position after uterine replacement.

It seems to us that magnesium sulfate might be the tocolytic medication of choice when uterine relaxation is required in this setting. Further, the use of prostaglandin $F_{2\alpha}$ or Prostin-15M should be considered to make the uterus contract well and to maintain it in preper position following repositioning.

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Reply

To the Editors:

We would like to thank Catanzarite et al. for communicating their experience with magnesium salfate and prostaglandins. The clinician, when confront ed with the obstetric emergency of an inverted uterus, has several options for therapy. The protocol outlined in their letter is certainly worthy of consideration.

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Cephalic replacement for shoulder dystocia

To the Editors:

Dr. Sandberg has described a "potentially r-v-lutionary" method for shoulder dystocia and named it the Zavanelli maneuver (AM J OBSTET GYNECOL 1935; 152:479-84). We would respectfully suggest that it might be a bit premature to place a proper name, that is, surname, on an unproven maneuver. Perhaps it should be called what it is: cephalic replacement

We would like to point out that since 1976 our women under our care have had a cephalic replacement for severe bilateral shoulder impaction. There were no unusual antenatal complications in these four were no except for one who was a noncompliant insuling pendent gestational diabetic patient. All were at term, and the pregnancies were not post dates. The labore were all normal except for one mildly protracted active phase. The second stages were not prolonged. Two indicated midforcep deliveries were performed for significant terminal fetal distress.

Before use of cephalic replacement or reduction, each patient was subjected to at least three distinctly different techniques for reduction of the severe Eateral shoulder dystocia. Once it became apparent that the situation was hopeless, a fetal scalp electroce was applied and subcutaneous terbutaline, 0.25 mg, sas administered to three of the patients. The head as easily replaced by use of constant but firm pressure with the palm of the right hand while the posterior vaginal wall was depressed with the left hand. The vertex was pushed as far cephalad as possible and hele in that position, usually zero station, by an assistan... The technique is very similar to the Hibbard maneuver except that the entire head was replaced into the vacina. Great pressure was not required, and the cephaic reduction was easy to carry out in every instance. The episiotomy was rapidly repaired.

All four infants initially experienced a prolonged teceleration to 60 to 70 bpm for 2 to 4 minutes, rapidly followed by a return to a normal baseline rate with good short- and long-term variability.

All four cesarean sections were routine for patients undergoing abdominal delivery at full dilation. The infants were delivered without difficulty, in good modition, with normal Appar scores at 1 and 5 minutes,

and with no injuries. The blood loss at the time of cesarean section was not unusual. There was no trauma to the lower uterine segment, bladder, vagina, cervix, or uterine vessels. Premature placental separation was not encountered in any case. There was no maternal morbidity, and all patients left the hospital in 5 to 6 days.

It needs to be emphasized that the technique should only be considered when continued persistence at vaginal manipulation will only lead to a predictably poor outcome for all concerned and when the infant has not been asphyxiated. Time is of the essence. Rapid cephalic reduction, tocolysis, repair of the episiotomy, and abdominal delivery seems to offer the infant, mother, and obstetrician the best outcome.

In summary, we feel that cephalic reduction is worthy of further investigation and reporting of all outcomes to see if this procedure will become a permanent part of our regimens for shoulder dystocia. In the past, rigid thinking dictated an all-or-none philosophy of vaginal delivery for shoulder dystocia at all costs. This experience with four patients suggests that a new alternative needs to be evaluated and that the proper naming of the maneuver should be left to the medical historians, if indeed it stands the test of time.

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To the Editors:

The recent article by Sandberg (AM J OBSTET GYNECOL 1985;152:479) describes "a potentially revolutionary method for the resolution of shoulder dystocia with replacement of the delivered fetal head back into the maternal pelvis followed by cesarean section." The single case reported was managed by Zavanelli in 1977 and only now has been brought to the attention of other obstetricians. In that Sandberg has reported Zavanelli's procedure and offered eponymic immortality, I also feel called upon to describe the work of another accoucheur.

In April, 1985, I was asked by Dr. James A. O'Leary to review his manuscript describing cephalic replacement and cesarean delivery of four infants with severe shoulder dystocia that could not be relieved by classical techniques. Each of the four infants was delivered in good condition with 5-minute Apgar scores of ≥8 and assumedly normal neurological follow-up. Dr. O'Leary's technique offers some refinement to that reported by Sandberg through the placement of a scalp electrode to assure satisfactory fetal heart rate pattern and administration of subcutaneous terbutaline for uterine relaxation before replacement of the fetal head.

My initial response to O'Leary's manuscript was one of incredulity, and I discouraged its submission for pub-

lication. Having read now that this procedure has been used also on the opposite coast, I urge all obstetricians to familiarize themselves with its possible use.

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Myotonic muscular dystrophy associated with ritodrine tocolysis

To the Editors:

We read with interest the paper by Sholl et al., "Myotonic muscular dystrophy associated with ritodrine tocolysis" (AM J OBSTET GYNECOL 1985;151:83-6). We would like to submit the following comments:

We know of three previous reports²⁻⁴ in which premature labor was treated with a chemically nearly identical β₂ receptor stimulator (fenoterol hydrobromide) and resulted in generalized myotonia. One mother was subsequently diagnosed to have myotonic dystrophy1; the other two women^{2,3} were found to have autosomal recessive generalized myotonia, a syndrome that is more common than generally realized.5-7 These reports indicate that ritodrine may aggravate myotonia of any cause, not only myotonia of patients with myotonic dystrophy; in each individual detailed diagnostic evaluation is therefore necessary. On the other hand, unmasking of myotonia by ritodrine during pregnancy should be a helpful and welcome hint to alert the obstetrician of the possibility of myotonic dystrophy in the mother and thus a baby at high risk.

We do not believe that ritodrine is a priori contraindicated in mothers with myotonic dystrophy if it is needed during premature labor; its "myotonic" effect is reversible and can be ameliorated with phenytoin.

Under the heading of Comment the authors indicate that ritodrine could have caused intracellular shifting of potassium and that this could have provoked the myotonia. Oral potassium replacement did not improve the myotonia. The resting muscle membrane potential is basically a potassium-potential; a higher intracellular potassium level would lead to a higher membrane potential and less myotonia, and a relative increase of the extracellular potassium level concentration should lead to a lower muscle membrane potential and higher propensity for myotonia. Indeed, it has been the clinical experience that increased serum potassium levels aggravate myotonia and potassium "loading" should be avoided in such patients.

The pathophysiologic mechanism as to how β -stimulating drugs cause worsening of myotonia is completely unknown. It is surprising that both β -stimulating and β -blocking agents^{8, 9} should aggravate myotonia; agents of both types are known to have membrane-stabilizing properties and theoretically should improve myotonia.

Finally, the title of the article was unfortunate and

misleading. As correctly concluded by the authors, the drug unmasked or precipitated the myotonia but did not cause the myotonic syndrome.

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Meconium aspiration syndrome

To the Editors:

Davis et al. have reported a mortality of 40% (12 of 30) in infants with meconium aspiration syndrome despite "appropriate" management (Fatal meconium aspiration syndrome occurring despite airway management considered appropriate. Am J Obstet Gynecol 1985;151:731-6). Pathologic descriptions are provided for the presence of meconium pigment in airways or macrophages. It is intuitively obvious that infants requiring "repetitive intubations" for pulmonary toilet would have traces of meconium present in the lung. What is not so obvious is the actual role of the meconium in these fatalities. Without having been provided data regarding the extent of pulmonary involvement on chest x-ray studies, the "thickness" of the meconium, and the incidence of air leak complications, we would

suggest that in at least nine of the 12 cases the true cause of death was persistent pulmonary hyperten ion of the newborn. Murphy et al. have demonstrate 1 hat this may be a primary persistent pulmonary hypercension of the newborn, that is, abnormal muscularization of the pulmonary vasculature, and that the outened therefore would be independent of the aspirated material. We have shown that in 18 infants with mecch am aspirations, survival was directly related to the severity of persistent pulmonary hypertension of the newborn, as well as to parenchymal pulmonary disease.

A clear distinction should be made between the gadromes of meconium aspiration (tachypnea, abnormal chest x-ray study, hypoxemia, and air leak) and perastent pulmonary hypertension of the newborn in Lacies who succumb, so that the appropriate pathologic factures will be analyzed and the true incidence of prinary persistent pulmonary hypertension of the newborn may be ascertained. While we agree that careful suctioning of the pharnyx is important, it is depressibly clear that, for a small subgroup at least, it may be of very little benefit.

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Reply ·

To the Editors:

The letter of Farrell and Hageman allows us to emphasize the thrust of our article—careful airway succtioning does not always prevent fatal respiratory disease in the newborn. Previous literature suggested that appropriate combined obstetric and pediatric airway management would prevent death from meconium aspiration syndrome. Review of this literature left the impression that death from respiratory disease following suction of meconium from the airways might be the result of inadequate physician management. A primary purpose of our report was to dispel this impression.

We agree with the statement that "the actual De of meconium" in the deaths we describe is unclear. Eastell and Hageman assert that as many as nine of the 12 deaths in our survey resulted from persistent puttonary hypertension of the newborn and suggest that a "clear distinction should be made between the syndromes of meconium aspiration . . . and persistent pul-

monary hypertension of the newborn". It is even more important to make a clear distinction between what we actually know and what we can only postulate about mecon:um aspiration and persistent pulmonary hypertension of the newborn. We know that many infants who die of persistent pulmonary hypertension of the newborn have hypertrophic pulmonary arterioles. 1-3 We know that nine of 10 infants who died with persistent pulmonary hypertension of the newborn and meconium aspiration in Murphy's series had pulmonary arteriolar hyperplasia.4 Of crucial importance, however, is the fact that one did not. It is of even greater importance that we have absolutely no data about the pulmonary arteriolar morphology in survivors of persistent pulmonary hypertension of the newborn. It may be that all infants with arteriolar hyperplasia are destined to die, whereas all those with spasm and no hyperplasia will survive. We suspect that some infants with hyperplastic changes do survive, but we do not (and may never) know this for certain. We also know that human meconium causes ventilation-perfusion mismatching, hypoxemia, and elevations in pulmonary artery pressure and resistance when instilled into normal rabbit lungs.5.6 We therefore suspect that meconium aspiration in humans may cause secondary persistent pulmonary hypertension of the newborn, even when the infant has normal pulmonary arterioles. This was apparently so in one of Murphy's cases. In addition, there is every reason to suspect that aspiration of meconium will exacerbate any preexisting tendencies toward development of persistent pulmonary hypertension of the newborn. Thus we hypothesize that meconium may actually cause persistent pulmonary hypertension of the newborn in some instances and exacerbate preexisting persistent pulmonary hypertension in others.

We submit that it is impossible to clinically distinguish between meconium aspiration syndrome-induced persistent pulmonary hypertension of the newborn and a similar preexisting hypertension exacerbated by meconium aspiration. All infants in our series with the diagnosis of meconium aspiration had radiographic evidence of meconium aspiration. Of the 12 who died, nine had clinical persistent pulmonary hypertension of the newborn. Of the eight with autopsies, four had clear evidence of meconium in the alveoli and small airways, accompanied by an inflammatory response, which surely contributed to the illness. Two other infants had numerous squamous cells in the alveoli. As far as we know, the role of aspirated squamous cells in neonatal pulmonary disease is unknown. However, since these cells were accompanied by an intra-alveolar inflammatory response, we doubt that the process was benign.

Primary persistent pulmonary hypertension of the newborn (defined as that being induced by pulmonary artericlar hyperplasia) can be diagnosed with certainty only by careful morphometric analysis of pulmonary artericles. Satisfactory morphometry is best achieved by perfusion fixation of lungs and is not routinely performed in most pathology laboratories. We did not per-

form such studies in our study. In fact, the pulmonary destruction in some of our cases was so extensive that accurate morphometric studies would have been impossible.

Thus clear distinction between primary persistent pulmonary hypertension of the newborn with meconium aspiration and meconium aspiration syndrome with secondary persistent pulmonary hypertension of the newborn is not possible in most centers. Our primary point remains—although aggressive airway suctioning is indicated for meconium staining, even the best job does not always prevent respiratory disease. In some cases this is because the aspirated material cannot all be removed, whereas in other cases the disease is primarily due to factors other than the presence of meconium or squamous cells. In either case the disease should not be considered as having resulted from physician error, as was implied by the previous literature.

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A sonographic sign for detection of Down's syndrome

To the Editors:

In their recent paper, "A sonographic sign for the detection in the second trimester of the fetus with Down's syndrome" (Benacerraf BR, Barss VA, Laboda LA. Am J Obstet Gynecol 1985;151:1078), Benacerraf et al. describe a sonographic finding that they noticed in association with Down's syndrome. This finding

is interesting and, if corroborated by others, would broaden the criteria for diagnostic amniocentesis and improve antenatal diagnostic accuracy. However, although the authors establish the differences between this finding and cystic hygroma, they do not address the possibility of coexisting nonimmune hydrops. An association between trisomy 21 and nonimmune hydrops is well documented in the literature, and the finding present in the picture can be part of this condition, which at a gestational age of 16 weeks may be difficult to diagnose.^{1,2}

In reading this report, we must assume that there were no other ultrasound findings, in which case it would have been interesting and helpful if the authors had given follow-up regarding the physical findings at birth. This information may have been helpful in establishing the pathophysiology of the ultrasound abnormality.

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Reply

To the Editors:

We are grateful for Dr. Castillo's interest in our paper, "A 30nographic sign for the detection in the second trimester of the fetus with Down's syndrome." Dr. Castillo is correct in his assumption that no other ultrasonographic findings were present in the fetuses that had the soft tissue thickening at the back of the neck in our report. In the cases of nonimmune hydrops that we have observed, whether in association with trisomy 21 or nct, there have been other findings, such as ascites or generalized scalp edema, to support a diagnosis of hydrops. The only sonographic finding in our three cases was the abnormal soft tissue at the back of the neck and no follow-up could be obtained at birth, since all three patients chose to terminate their pregnancies. As noted in the paper, however, soft issue thickening at the back of the neck is a well-known physical finding in neonates with Down's syndrome, and according to the ped atric literature, it is present in 80% of babies with the syndrome.

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Stimulation of plasma prorenin by gonadoarcpic hormones

To the Editors:

As a follow-up to our report (AM J OBSTET GYNECOL 1985;153:514-9), entitled "Plasma prorenin in first-trimester pregnancy: relationship to changes in numan chorionic gonadotropin," we would like to report the following.

A 25-year-old woman received the oral gonadot opic hormone-stimulating compound Clomid (clomphene citrate), 50 mg, on days 3, 4, and 5 of the mensional cycle, followed by Pergonal [a combination of luteinizing hormone (LH) and follicle-stimulating hormone (FSH), 75 units intramuscularly, two times] on days 6 to 10; she then received 10,000 units of human chorionic gonadotropic hormone (hCG) on day 11 to induce ovulation.

Plasma prorenin increased to 50% above base in a after Clomid (Fig. 1), to 300% above baseline after Fergonal, and to 1000% above baseline after hCG (ca-12). Active renin also increased but reached its peak (£00% above baseline) on day 16, at a time when prorecin had already fallen to 50% of maximum. Estradiol eached a peak on day 9; a second peak occurred on lay 16, the same day as the progesterone and active renin peaks.

Thus plasma prorenin increased before active rerin; it reached its peak level before active renin, and its relative increase was much greater than that of active renin. The time course of the change in active edin is consistent with a response to the diuretic effect of induced changes in progesterone. In sharp contrast, the greatest increase in prorenin occurred soon after hCG administration, which seemed to have little effect on active renin. The peak of plasma prorenin was as high as the highest level we have found in women during the first trimester of pregnancy (AM J OBSTET GINECOL 1985;153:514-9) and the response was greater that has been previously reported for any other stimuli known to affect prorenin.

Immunoreactive renin (which could be either active renin or prorenin or both) is present in the LH-containing cells of the anterior pituitary. However, Clomid, which releases LH from the anterior pituitary, barely affected prorenin when compared with exceenous gonadotropic hormone administration, which had a marked effect. The results therefore suggest that LH (or FSH) and hCG directly stimulated prorein biosynthesis or release and support the view that he rise in prorenin that occurs soon after conception may indeed be the result of stimulation by hCG.

Since we submitted our report to this JOURNAL we have also observed cyclic changes in plasma protein, independent of active renin levels, during the menstrual cycle. Prorenin increased about twofola at the time of the LH peak. In contrast, active renin usually remained stable until several days later, when it increased in association with progesterone. Altagether, the results are consistent with the hypothesis that prorenin is synthesized and secreted from the ovaries in

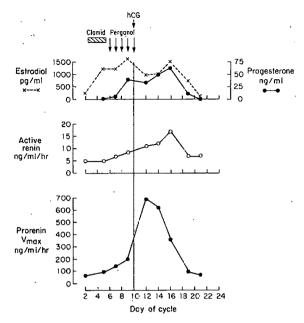


Fig. 1. Effects of Clomid (50 mg on days 3 to 5), Pergonal (75 units intramuscularly daily, two times, on days 6 to 10), and hCG (10,000 units on day 10) on plasma prorenin, active renin, estradiol, and progesterone. Prorenin was calculated by subtracting active renin¹ from total renin, which was measured after solid phase trypsin activation.² To estimate changes in prorenin independent of changes in renin substrate concentration, the prorenin concentration at excess substrate concentration (*Prorenin V*_{max}) was calculated from the plasma prorenin and renin substrate levels with the use of the Michaelis-Menten equation and a Michaelis-Menten coefficient of 2700 ng/ml.³ Estradiol and progesterone were measured by radio-immunoassay with the use of kits from Serono Diagnostics.

response to LH or hCG. These findings therefore suggest that prorenin plays a role in reproductive physiology in addition to its renal role as a precursor of active plasma renin.

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Initial instruction in pelvic examination

To the Editors

I am a little behind with my postgraduate reading and have only just picked up the January I, 1985, issue of the Gray Journal. In it I found an excellent article, "Initial instruction in the pelvic examination in the United States and Canada, 1983" by Beckmann et al. (Am J Obstet Gynecol 1985;151:58). This article provides further support for the concept that the use of "Teaching Associates" is by far the best method of introducing students to that delicate area, the pelvic examination.

I have two questions about the use of the English language in this paper. In the first paragraph, reference is made to "... work with stimulated patients." One wonders in what way these patients might have been stimulated. Were they "psyched up" for this encounter with a sort of feminist zeal? Or were they perhaps stimulated to participate by the prospect of appropriate remuneration, coupled with academic achievement? On the other hand, is it possible that the word in question should have been "simulated," suggesting that the subjects were pretending to be patients?

In the last paragraph on the same page, the sentence reads, "A questionnaire was sent to all medical schools seeking this information." Was the questionnaire, in fact, sent only to those schools who were looking for such data, as the sentence suggests? Should the sentence have read "A questionnaire, seeking this information, was sent to all medical schools"?

Language is not a static entity. Change is inevitable

and even healthy. However, deterioration of a language is to be resisted, especially when it leads to confusion on the part of the reader as to the meaning of the author's message.

I would appreciate your clarifying the above points for me.

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Reply

To the Editors:

I appreciate the opportunity to respond to Dr. Coopland's letter concerning the article "Initial instruction in the pelvic examination in the United States and Canada, 1983." Dr. Coopland is correct that a typographical error resulted in the printing of the word "stimulated" when the correct word was "simulated." Simulated pa-. tients have been used in medical education for some time, and Dr. Coopland is correct that the concept of the simulated patient does not strictly apply to the gynecologic teaching associate. In the case of the simulated patient, individuals are trained to duplicate the signs and symptoms of diseases; gynecologic teaching associates, in contrast, teach normal anatomy and physiology and do not, as a rule, simulate pathophysiologic conditions. This is a basic concept and as such, demonstrates one of the strengths of the gynecologic teaching associate in the instruction in the pelvic examination.

Dr. Coopland is also correct that the sentence concerning the distribution of the questionnaire should have included commas as follows: "A questionnaire, seeking this information, was sent to all medical schools."

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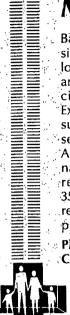
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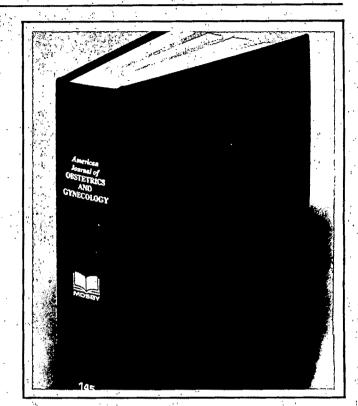
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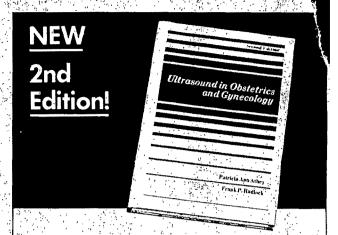
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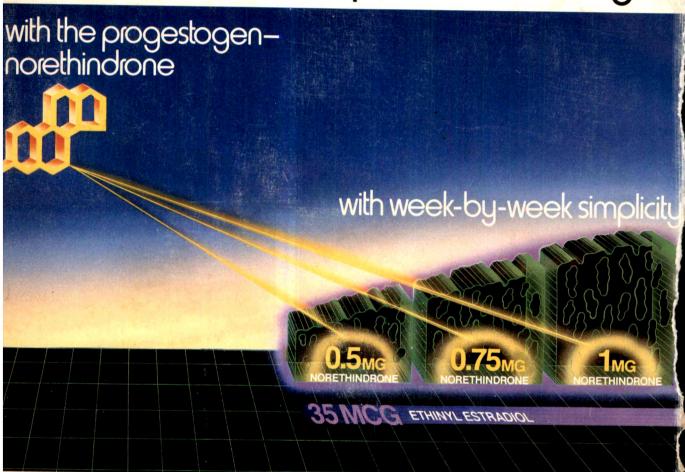
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